MULTIMODAL FLAVOUR PERCEPTION:

The impact of sweetness, bitterness, alcohol content and carbonation level on flavour perception

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ABSTRACT

Flavour perception of food and beverages is a complex multisensory experience involving the gustatory, olfactory, trigeminal, auditory and visual senses. Thus, investigations into multimodal flavour perception require a multidisciplinary design of experiments approach. This research has focussed on beer flavour perception and the fundamental interactions between the main flavour components – sweetness, bitterness (from hop acids), alcohol content and carbonation level. A model beer was developed using representative ingredients which could be manipulated to systematically vary the concentration of the main flavour components in beer and was used in the following experiments.

Using a full factorial design, the physical effect of ethanol, CO_2 and hop acid addition was determined by headspace analysis and in-nose expired breath (in-vivo) measurements. Results from headspace and in-vivo methods differed and highlighted the importance of in-vivo measures when correlating to sensory experience. Ethanol and CO_2 significantly increased volatile partitioning during model beverage consumption. The effects of ethanol and CO_2 appeared to be independent and therefore additive, which could account for up to 86% increase in volatile partitioning. This would increase volatile delivery to the olfactory bulb and thus potentially enhance aroma and flavour perception. This was investigated using quantitative descriptive analysis. Results showed that CO_2 significantly impacted all discriminating attributes, either directly or as a result of complex interactions with other design factors. CO_2 suppressed the sweetness of dextrose and interacted with hop acids to modify bitterness and tingly perception. Ethanol was the main driver of complexity of flavour

and enhanced sweet perception. In a first study of its kind, the impact of CO_2 on gustatory perception was further investigated using functional magnetic resonance imaging (fMRI) to understand cortical response. In addition, classification of subjects into PROP taster status groups and thermal taster status groups was carried out. Groups were tested for their sensitivity to oral stimuli using sensory techniques and for the first time, cortical response to taste and CO_2 was investigated between groups using fMRI techniques and behavioural data. There was no correlation between PROP taster status and thermal taster status. PROP taster status groups varied in their cortical response to stimuli with PROP super-tasters showing significantly higher cortical activation to samples than PROP non-tasters.

The mechanism for thermal taster status is not currently known but thermal tasters were found to have higher cortical activation in response to the samples. The difference in cortical activation between thermal taster groups was supported by behavioural data as thermal tasters least preferred, but were more able to discriminate the high CO_2 sample than thermal non-tasters.

This research has provided in-depth study into the importance of flavour components in beer. It advances the limited data available on the effects of CO_2 on sensory perception in a carbonated beverage, providing sound data for the successful development of products with reduced ethanol or CO_2 levels. The use of functional magnetic resonance imaging has revealed for the first time that oral CO_2 significantly increases activation in the somatosensory cortex. However, CO_2 seemed to have a limited impact on activation strength in 'taste' areas, such as the anterior insula. Research comparing data from PROP taster status groups and

thermal taster status groups has given insight into the possible mechanisms accounting for differences in oral intensity of stimuli.

PREFACE

Much is known about the impact of individual components on beer quality but only limited published research exists concerning the interactions between different sensory stimuli. This research has taken a scientifically controlled approach to investigate the physical and perceptual interactions between the primary flavour components in beer; sweetness, bitterness, alcohol and carbonation and their affect on flavour perception. In order for the individual components to independently manipulated, a model beer system was developed that systematically varied in bitter and sweet components, alcohol content and carbonation level. Interactions between components were investigated at three levels; physico-chemically, sensorially and cortically. Chemical interactions between matrix components in the solution may impact flavour perception independent of peripheral or cortical interactions. Such interactions were investigated and then validated by human sensory assessments. The resultant data was used to construct mathematical models to represent the contribution of the various stimuli and their interactions to the sensory properties of the beer system. In humans, investigations beyond this point present a significant number of ethical and technical difficulties. Fortunately cutting edge neuroimaging techniques such as Functional Magnetic Resonance Brain Imaging allow scientific advancement to further the understanding of flavour perception. This method was employed using innovative sample delivery techniques to investigate the interaction between taste and carbonation. Little data exists in the literature concerning the effects of carbonation on flavour perception presumably due to the difficult nature of creating and working with pressurised systems. As a result the pathways responsible for CO_2 perception in combination with taste

stimuli are not fully understood. Using the approach described above, the research aims to uncover the effects of carbonation in combination with other primary flavour components in beer on flavour perception and the possible mechanisms responsible for the interactions.

In addition, the population varies in their sensitivity to oral stimuli which may alter perception adding another layer of complexity multimodal flavour research. Two markers of genetic oral sensitivity, PROP taster status and the newly discovered thermal taster status were investigated sensorially and cortically. Results provide novel insight into the possible mechanisms contributing to oral sensitivity. This fundamental research will provide understanding of the chemical and perceptual sensory interactions in a model beer system and some understanding of the mechanisms behind them. It will provide direction and a sound basis for follow-on studies which address the understanding consumer perception and differences between the population's oral sensitivity.

THESIS STRUCTURE

Chapter 1 gives a general introduction to the individual sensory systems and interactions between them. It introduces the experimental approach taken and methods employed. Chapter 2 details the development of the model beer system which was used in subsequent experimental chapters. Chapters 3, 4, 5 and 6 detail experimental work undertaken. Each chapter includes an introduction with a detailed literature review specific to that investigation; followed by materials and methods, results, and in depth discussion sections. Chapter 3 reports an investigation into

the physico-chemical interactions between the components in the beverage matrix. Chapter 4 details the sensory evaluation of the model beverages. Chapter 5 focuses on investigating genetic differences in oral sensitivity using sensory sensitivity measures and served as a screening tool for subjects selected for participation in the following study. Chapter 6 reports the experimental results from conducting an fMRI study to investigate the cortical effect of carbonation on taste perception and differences in cortical activity between different population groups. Finally, Chapter 7 provides an overview of major findings from all experimental work conducted, general conclusions and further work.

Chapter 1

1. GENERAL INTRODUCTION

Despite a reduction in the consumption of alcohol across the UK population, beer continues to be a popular alcoholic beverage, worth more than any other drink type (in sales value), (Mintel 2007). The discovery of beer is said to be the result of widespread cereal grain farming at around 10,000 BC (Hornsey 2003). It was soon discovered that when the grains were mixed with water they began to sprout and taste sweet (now known as malting). After being left for a few days the mixture became fizzy and pleasantly intoxicating (fermentation by wild yeasts). Presumably many years of trial and error has improved the quality and now modern man understands the science behind beer production a vast variety of products are available. Each product varies in either the ingredients used or the production method, but all have primary flavour elements; alcohol, bitterness and carbon dioxide (Meilgaard 1975; Meilgaard 1982). The concentration of each of these elements varies depending upon the style of beer to be produced. Sweetness results from unfermentable residual carbohydrates comprising of a complex mixture of dextrins. Bitterness results from hop addition; whilst both alcohol and carbon dioxide are by-products of yeast fermentation. The brewing process produces a large number of volatile compounds and whilst each on their own does not dominate the flavour, they combine to contribute to the beer's secondary flavour components (Meilgaard 1975). In 1979, Meilgaard et al introduced the beer flavour wheel (**figure 1.1**) as a unified system to communicate flavour terminology within the industry.

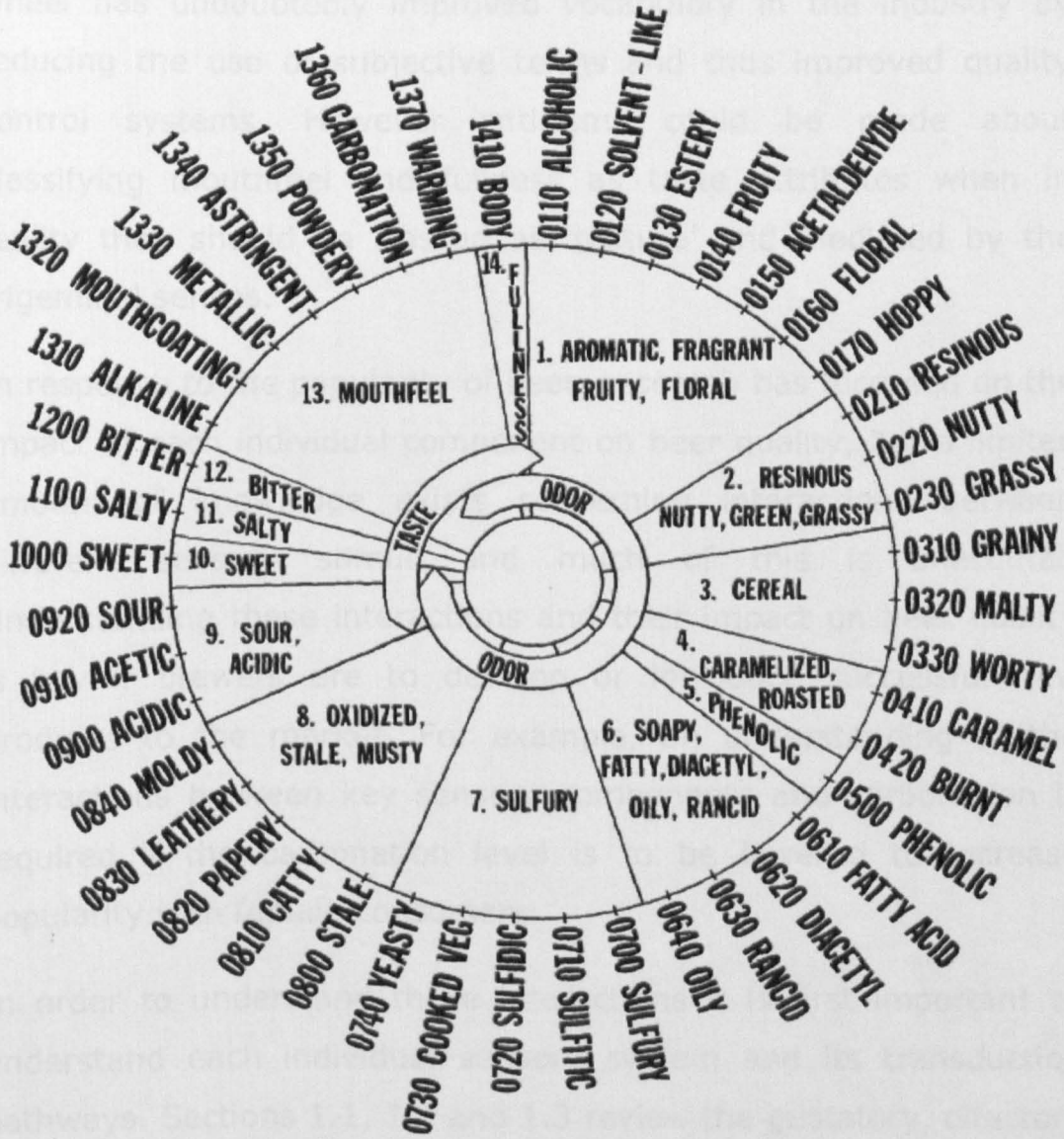

Figure 1.1: The Flavour wheel, showing class terms & first tier terms.

Source: (Meilgaard, Dalgliesh et al. 1979)

The wheel consists of 14 class terms under which first tier terms are grouped. There are many compounds which fall into the categories and these are named and defined as separately identifiable flavour notes in separate second tier term tables. Reference standards (where available) were also published alongside these terms to help define first tier terms and aid in specialised panel training. The development of the beer flavour

wheel has undoubtedly improved vocabulary in the industry by reducing the use of subjective terms and thus improved quality control systems. However criticisms could be made about classifying mouthfeel and fullness as taste attributes when in reality they should be classed as 'texture' and mediated by the trigeminal senses.

In response to the popularity of beer, research has focussed on the impact of each individual component on beer quality, but a limited amount of knowledge exists concerning interactions between different sensory stimuli and much of this is anecdotal. Understanding these interactions and their impact on beer quality is key if brewers are to develop or introduce successful new products to the market. For example, an understanding of the interactions between key sensory components and carbonation is required if the carbonation level is to be lowered to increase popularity with female consumers.

In order to understand these interactions it is first important to understand each individual sensory system and its transduction pathways. Sections 1.1, 1.2 and 1.3 review the gustatory, olfactory and trigeminal systems respectively and section 1.4 considers interactions between modalities.

1.1 THE GUSTATORY SYSTEM

Taste buds are distributed across three types of papillae on the tongue, fungiform, foliate and circumvallate (**figure 1.2**). When a food or drink is consumed, the sapid molecules dissolve into the saliva and enter the taste pore of the taste bud which is densely packed with taste receptor cells (TRCs). Each taste bud contains

between 50-150 TRC's which are specialised epithelial cells that respond to all five taste modalities (Hoon, Adler et al. 1999; Adler, Hoon et al. 2000; Chandrashekar, Hoon et al. 2006).

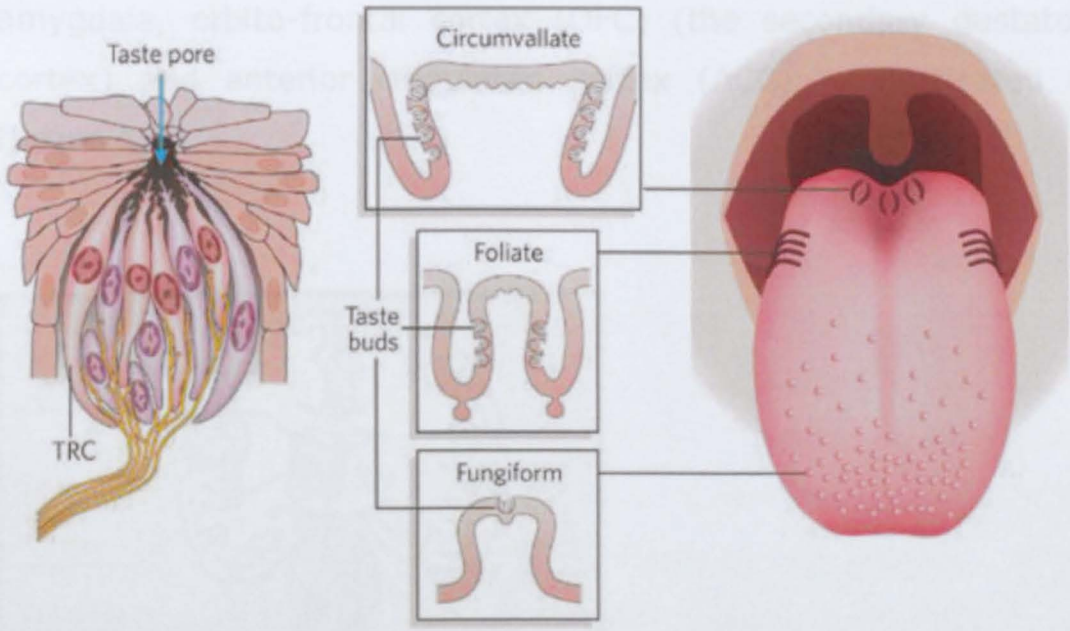

Figure 1.2: Taste receptor anatomy and location of papillae on tongue. Source: (Chandrashekar, Hoon et al. 2006)

When activated by a tastant, TRCs depolarise, leading to neurotransmitter release and projection of action potentials along the axons of the sensory nerves that innervate the tongue and soft palate (Lindemann 2001). The chorda tympani branch of the facial nerve (cranial nerve VII) innervates the anterior two thirds of the tongue where the fungiform papillae are located. The glossopharyngeal nerve (cranial nerve IX) innervates the remaining third where the foliate and circumvallate papillae are situated and the vagus nerve (cranial nerve X) innervates the epiglottis and larynx (Chandrashekar, Hoon et al. 2006; Sugita 2006). Together these nerves send taste information along the brainstem to the

nucleus of the solitary tract (NTS) and onwards to the thalamus and the gustatory cortex (Boucher, Simons et al. 2003). The primary gustatory cortex consists of the insula cortex and the frontal operculum (Kobayakawa, Endo et al. 1996) which covers part of the insula. Taste projections may then continue to the amygdala, orbito-frontal cortex (OFC) (the secondary gustatory cortex) and anterior cingulated cortex (ACC) as illustrated by **figure 1.3**.

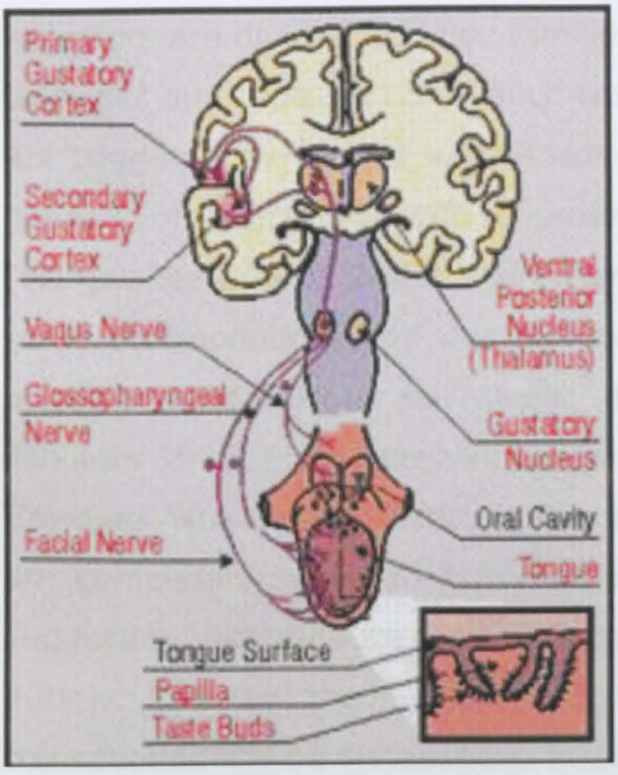

Figure 1.3: Taste transduction and associated cortical areas. Source: http://quizlet.com/4414696/biopsych-midterm-2-flash-cards/

There are two opposing views of how different tastes are detected. The view that TRCs are individually tuned to respond to specific taste qualities with individually tuned nerve fibres is called the labelled-line theory. The across-fibre model proposes that TRC's

can be either broadly or individually tuned to respond to multiple taste modalities because the same afferent fibre carries information for more than one taste modality (Chandrashekar, Hoon et al. 2006). There are five primary tastes, sweet, bitter, salt, sour and umami each taking different taste receptor pathways to the gustatory cortex (Lindemann 2001). Salt and sour compounds are believed to enter the taste cell via specialised ion gated membrane channels which allows direct entry of Na^+ and H^+ on the surface of the cell (Chandrashekar, Hoon et al. 2006). Sweet, bitter and umami tastes are transduced by specific receptors. These specific receptors are divided into two families, type 1 (T1R) which respond to sweet and type 2 (T2R) which respond to bitter. The receptors are triggered by contact with Gusducin, a G-protein with subunits capable of interacting with G-protein-coupled-receptors (GPCRs) (Margolskee 2002). Sweet and bitter transduction pathways share some commonalities and this has lead to much research into the possible mechanisms for sweet and bitter taste interactions (Walters 1996; Margolskee 2002; Talavera, Yasumatsu et al. 2008). However, Nelson *et al* (2001) has shown that cells expressing T2Rs are completely segregated from those expressing T1R receptors, suggesting different transduction pathways (Nelson, Hoon et al. 2001). Due to their importance in beer, sweet and bitter transduction will be discussed in detail in the following sections.

1.1.1 Sweetness perception

Sweet taste perception is mediated by a family of GPCRs, the T1Rs. It has been proposed that a combinational arrangement of two members of a family of three receptors; T1R1, T1R2 and T1R3 may be sufficient to detect all sweet compounds (Nelson, Hoon et al.

2001; Li, Staszewski et al. 2002). Experiments using laboratory mice and genome sequencing led to the breakthrough that two taste receptor proteins, T1R2 and T1R3, form a complex to produce a GPCR that demonstrates broad selectivity by responding to many structurally different sweet molecules (Hoon, Adler et al. 1999; Nelson, Hoon et al. 2001; Li, Staszewski et al. 2002). These findings suggest that the T1R2+3 complex is the predominant sweet taste receptor (Nelson, Hoon et al. 2001; Chandrashekar, Hoon et al. 2006).

Sugars activate GPCRs which stimulate the enzyme adenylyl cyclase to generate cAMP, a second messenger molecule (Meyers and Brewer 2008). This can then either act directly, to cause cation influx through cNMP-gated channels resulting in neurotransmitter release, or indirectly, to activate the protein kinase. This results in phosphorylation of basolateral K^+ channels and closure of K^+ channels causing depolarisation of the taste cell and Ca^{2+} influx triggering neurotransmitter release (Margolskee 2002). Artificial sweeteners stimulate the enzyme $PLC\beta_2$ which forms secondary messengers IP_3 and DAG which releases Ca^{2+} from internal stores resulting in depolarisation of the TRC and neurotransmitter release (Margolskee 2002; Meyers and Brewer 2008).

1.1.2 Bitterness Perception

To date, gene studies have identified ~25 potential bitter receptors belonging to the T2R family which are responsible for bitter taste when coupled to the G-protein, gustducin (Adler, Hoon et al. 2000; Chandrashekar, Mueller et al. 2000; Matsunami, Montmayeur et al.

2000; Meyerhof, Behrens et al. 2005; Behrens, Foerster et al. 2007). When activated they mediate one of two responses in the TRCs. Activated α-gustducin stimulates the enzyme PDE to hydrolyse cAMP which may decrease cNMP, with the subsequent steps in this pathway remaining uncertain (Margolskee 2002; Behrens and Meyerhof 2006). The second transduction pathway involves activated $PLC\beta_2$ to generate IP_3. Both pathways result in elevated intracellular levels of Ca^{2+} and neurotransmitter release (Behrens and Meyerhof 2006).

Bitter taste perception is complex and this is further complicated by the genetic variation associated with polymorphisms in several T2R genes. The variability in sensitivity to the chemical compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) is explained by the TAS2R38 gene and is known as PROP taster status. PROP tastes bitter but the intensity is dependent upon a person's 'taster status'. Those who can taste PROP intensely are classified as PROP tasters and those who cannot are categorised as PROP non-tasters. The possibility that PROP taster status effects overall bitter taste sensitivity and the sensitivity of other compounds is discussed further in Chapter 5. Functional expression studies have been used to investigate the bitter receptors that respond to hop derived compounds. Results found that various combinations of three bitter taste receptors, hTAS2R1, hTAS2R14 and hTAS2R40, were activated by the 15 hop derived compounds investigated (Intelmann, Batram et al. 2009). This work adds to evidence that some bitter receptors are broadly tuned as they can be activated by chemically different compounds (Adler, Hoon et al. 2000; Intelmann, Batram et al. 2009) but discrimination between

them is difficult, thus supporting the across fibre pattern theory of taste detection.

1.2 THE OLFACTORY SYSTEM

When volatile molecules are in the gaseous phase they can enter the nasal cavity via the orthonasal (via sniffing) or the retronasal (during consumption) routes to interact with the olfactory neurons in the olfactory bulb (**figure 1.4**).

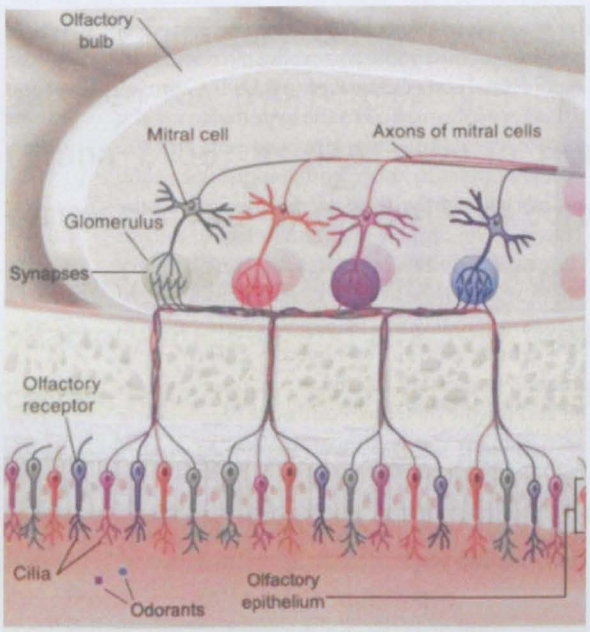

Figure 1.4: Anatomy of the olfactory bulb. Source: (Anon 2010)

Once the volatiles have entered the nasal cavity, they become dissolved in a mucus layer surrounding the cilia. Cilia project from the olfactory receptors and contain the olfactory receptor proteins which are the active sites for olfaction (Goldstein 1999). Odorant molecules reach these active sites directly via inhaled air or by binding to an olfactory binding protein (OBP) (Snyder, Sklar et al. 1989), both of which activate the olfactory G-protein on the inside

of the olfactory neuron which activates the lyase enzyme, alenylate cyclase to convert ATP to cAMP (Buck and Axel 1991). cAMP opens cyclic nucleotide gated ion channels which allows Ca^{2+} and Na^{+} influx, depolarising the receptor neuron and sending input directly to the glomeruli in the ipsilateral olfactory bulb (Brand 2006). Each glomeruli receives information from one particular type of receptor (Buck and Axel 1991) and sends it to the mitral cells (Goldstein 1999). An action potential transmits the signal along the olfactory nerve to the primary olfactory cortex (Buck 2004). Direct ipsilateral signalling by second order neurons from the olfactory bulb to the primary olfactory cortex (with only few contralateral connections between the two hemispheres) is unique to the olfactory system (Brand 2006) as illustrated in **figure 1.5**. The primary olfactory cortex consists of the piriform cortex, anterior cortical amygdaliod nucleus, periamygdaliod cortex, entorhinal cortex, anterior olfactory nucleus and olfactory tubercle. From the primary olfactory cortex, olfactory information projects to the secondary olfactory cortex consisting of the insula, ventral striatum, orbito-frontal cortex, hypothalamus and hippocampus.

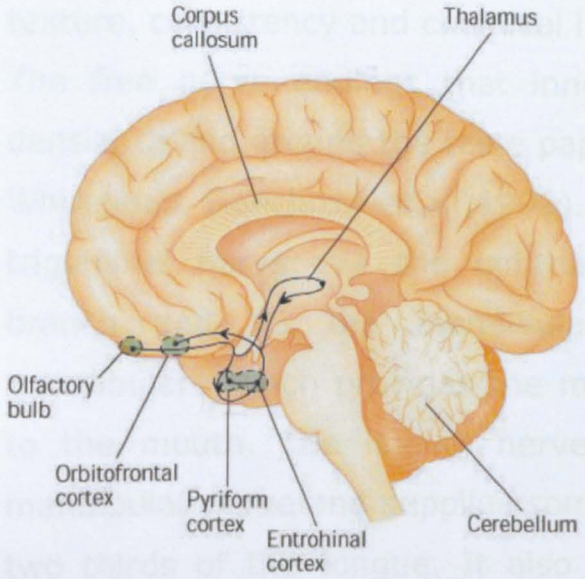

Figure 1.5: Direct signalling to piriform cortex and entrohinal cortex (primary olfactory cortex) before projecting to the orbitofrontal cortex and thalamus (secondary olfactory cortex). Source: http://mva.me/educational/brain_areas/smell.jpg

Each olfactory receptor is capable of detecting multiple odorants and each is detected by multiple receptors (Buck 2004). There are ~350 olfactory receptors used in a combinational manner to encode odour identities resulting in detection of over 100,000 aroma compounds (Buck 2004). While humans can detect a vast array of aromas, they lack discrimination ability both in identification and detecting differences in intensity (Desor and Beauchamp 1974; Laing and Francis 1989; Laska and Hudson 1992). The former can be increased by training (Desor and Beauchamp 1974) as it relies on the memory which can be improved.

1.3 THE TRIGEMINAL SYSTEM

The trigeminal system provides tactile, proprioceptive and nociceptive afference from the mouth, providing information on the

texture, consistency and chemical irritation of foods and beverages. The free nerve endings that innervate the tongue are high in density in and around the taste papillae (Nagy, Goedert et al. 1982; Whitehead, Ganchrow et al. 1999). There are three branches of the trigeminal nerve; V1 the ophthalmic branch, V2 the maxillary branch and V3 the mandibular branch (**figure 1.6**). The mandibular branch provides the main source of nerve innervation to the mouth. The lingual nerve is the largest branch of the mandibular nerve and supplies somatic innervations to the anterior two thirds of the tongue. It also carries multiple types of nerve fibres, such as those from the chorda tympani which innervate taste.

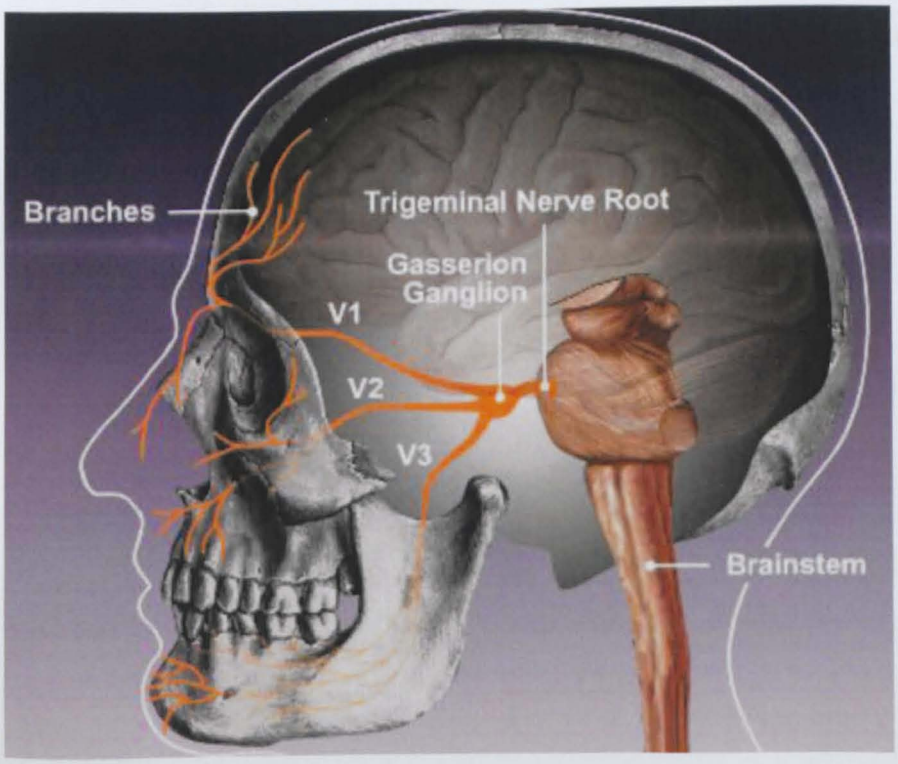

Figure 1.6: The three branches of the trigeminal nerve. Source: (Kaufmann, Patel et al. 2001)

Trigeminal information from the face is carried by first order neurons to the principle nucleus of the trigeminal complex (Brand 2006) where secondary fibres cross the midline and ascend along the medial lemniscal pathway via the trigeminal lemniscus to the contralateral thalamus (Abdi 2002; Brand 2006). Third order neurons project to the VPM (ventral posterior medial) nuclei in the thalamus and to the primary (SI) and secondary (SII) somatosensory cortices (Abdi 2002; Carstens, Carstens et al. 2002; Brand 2006) located in the postcentral gyrus as shown in **figure 1.7**. SII receives input mainly from SI and directly from the thalamus. It projects back to SI, the primary motor cortex and the posterior insula (Youell, Wise et al. 2004). SI and SII process physical and discrimination aspects of somatosensory information from the whole body, with information from the oral cavity being processing in the most ventral part of SI, just lateral to SII.

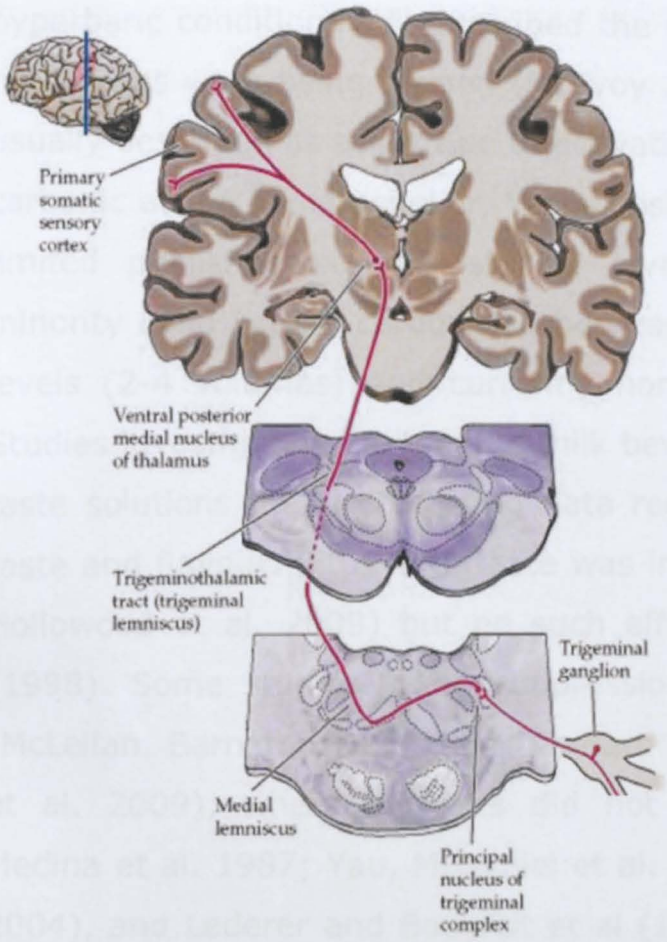

Figure 1.7: The trigeminal pathway. Source: (Purves, Augustine et al. 2001)

1.3.1 Carbonation perception

CO_2 perception is complex, involving excitatory and inhibitory processes in the oral somatosensory system (Green 1992). It is well accepted that CO_2 acts on oral trigeminal receptors via a dual mechanism of action. The presence of bubbles bursting in the mouth activates mechanoreceptors while the conversion of CO_2 to carbonic acid via carbonic anhydrase elicits a tingly response activating nociceptors. These mechanisms have been decoupled via the use of carbonic anhydrase inhibitors to block the conversion of CO_2 and reduce the intensity of carbonation (Simons, Dessirier et al. 1999; Dessirier, Simons et al. 2000) and by inhibiting bubble formation via use of a hyperbaric chamber. Subjects under

hyperbaric conditions still described the tingle of CO_2 even though no bubbles were being formed (McEvoy 1998). The taste of CO_2 is usually described as acidic due to activation of acid sensing cells by carbonic acid (Chandrashekar, Yarmolinsky et al. 2009). There are limited published sensory studies investigating carbonation, a minority used typical carbonated beverages at representative CO_2 levels (2-4 volumes) and currently none have included alcohol. Studies investigating carbonated milk beverages, juices and simple taste solutions found conflicting data regarding effects of CO_2 on taste and flavour. Bitter aftertaste was increased by CO_2 (Hewson, Hollowood et al. 2009) but no such effect was found by Cowart (1998). Some studies found suppression of sweetness with CO_2 (McLellan, Barnard et al. 1984; Cowart 1998; Hewson, Hollowood et al. 2009), whereas others did not (Cometto-Muniz, Garcia-Medina et al. 1987; Yau, McDaniel et al. 1989; Prescott, Soo et al. 2004), and Lederer and Bodyfelt et al (1991) found a suppression effect in only one of the four flavoured carbonated milks investigated. The same study found a suppression effect of CO_2 on cooked milk flavour (Lederer, Bodyfelt et al. 1991), whereas, flavour intensity was increased by CO_2 addition in a study investigating blueberry milk (Yau, McDaniel et al. 1989). Fruity apple aroma (not flavour) was not significantly altered by carbonation in a study by McLellan and Barnard et al (1984). Cowart (1998) suggested that CO_2 impacts on taste perception but also alters the taste 'quality' and therefore results may be dependent upon the combination and levels of tastants present in the specific beverage. It is possible that these effects are the result of chemical interactions between tastant and irritant at the periphery, but they may also be due to cortical convergence of signals (Verhagen and Engelen 2006). Consequently further

research with commonly carbonated beverages at appropriate CO_2 levels is important to further this understanding. In addition, research investigating interactions between taste and CO_2 in beer are needed as there are currently no studies reporting this.

1.3.2 Ethanol perception

Ethanol is a complex stimulus which acts on multiple modalities (Green 1988; Kiefer and Morrow 1991; Mattes and DiMeglio 2001; Cometto-Muniz and Abraham 2008) and ethanol has been found to interact with beverage components to modify sensations. For example ethanol has been shown to contribute to the sweetness of sucrose and the bitterness of quinine (Martin and Pangborn 1970), the astringency and bitterness of tannins (Fontoin, Saucier et al. 2008), irritation (Prescott and Swain-Campbell 2000), hotness (Jones, Gawel et al. 2008) and perceived complexity of wine (Meillon, Viala et al. 2010) as well as aroma (Goldner, Zamora et al. 2009). In sensory studies, the taste of ethanol has been found to include both sweet and bitter components depending on the concentration (Wilson, Obrien et al. 1973; Scinska, Koros et al. 2000; Mattes and DiMeglio 2001). Neuronal taste response of ethanol, investigated in vitro using the rhesus monkey (Hellekant, Danilova et al. 1997), rats (Lemon, Brasser et al. 2004) and mice (Brasser, Norman et al. 2010) supports evidence that ethanol stimulates fibres which respond best to sweet compounds (sweet-best fibres) and that central processing follows a similar pathway to sucrose. Ethanol stimulation of the trigeminal system seems to be multifaceted, evoking both chemical irritation pathways and mechanoreceptors (Green 1991; Trevisani, Smart et al. 2002; Ellingson, Silbaugh et al. 2009; Goldner, Zamora et al. 2009).

Ethanol is said to contribute to mouthfeel characteristics with the flavour being described as solvent-like (Langstaff and Lewis 1993).

1.4 MULTIMODAL FLAVOUR PERCEPTION

Current understanding is that flavour perception is multimodal where-by information detected at the receptors located at each of the five senses has the capacity to merge and interact physically in the product matrix itself, at the periphery or centrally in the brain to influence sensation (Verhagen and Engelen 2006). Physical interactions between aroma compounds and other components in foods and beverages have been widely researched and will be discussed further in Chapter 2. Sensory integration from independent modalities (gustatory, olfactory, trigeminal, visual and auditory) all contribute to give a final percept of flavour. Investigations using sensory evaluation and magnetic resonance imaging methods provide understanding of interactions between different modalities. Within-modal interactions, such as taste-taste interactions, bring about either an enhancement or a suppression effect dependent upon the tastant and the concentration (Breslin 1996; Keast and Breslin 2003). Cross-modal interactions, such as the interaction between anatomically separate organs are well documented (Dalton, Doolittle et al. 2000; Hort and Hollowood 2004; Pfeiffer, Hollowood et al. 2005). Dalton and Doolittle et al (2000) used sub-threshold levels of saccharin and benzaldehyde to demonstrate the central neural integration of a congruent taste and aroma. When the sub-threshold stimuli were presented simultaneously they could be detected demonstrating taste-aroma interactions between congruent pairings. However, this was not the case when incongruent stimuli (MSG and benzaldehyde) were

presented together (Dalton, Doolittle et al. 2000). Interactions between the olfactory and trigeminal systems have been investigated. Trigeminal fibres innervate the olfactory epithelium and seem to respond to olfactory stimuli but may also modify the olfactory response (Cain and Murphy 1980; Prescott and Stevenson 1995). A study by Laska (1997) showed that chemical compounds with a strong trigeminal component can be discriminated and described by olfaction alone in anosmic subjects suggesting that the trigeminal system contributes to the perception of odour (Laska, Distel et al. 1997). However, interactions between the two systems are not clearly established due to the complex nature of the interactions which appear to differ dependently of molecules, intensity or context of inhalation (Brand 2006). Gustatory and trigeminal systems have also been found to interact. Temperature (Moskowit.Hr 1973; Bartoshuk, Rennert et al. 1982), irritation (Prescott, Allen et al. 1984; Lawless, Rozin et al. 1985; Cometto-Muniz, Garcia-Medina et al. 1987) and texture (viscosity) (Cook, Hollowood et al. 2002) have all been found to interact with taste. The influence of temperature will be discussed further in **Chapter 5**. The influence capsaicin has on taste is the most researched irritant and generally produces a suppression effect (Lawless and Stevens 1984; Prescott, Allen et al. 1984; Lawless, Rozin et al. 1985; Simons, O'Mahony et al. 2002; Simons, Boucher et al. 2003).

Many psychophysical studies have investigated perceptual multimodal interactions and have alluded to possible mechanisms but few have investigated these mechanisms in humans. fMRI techniques have been used to study taste-aroma interactions (Cerf-Ducastel and Murphy 2001; Marciani, Pfeiffer et al. 2006; Rolls, Critchley et al. 2010) and taste-tactile interactions (de Araujo

and Rolls 2004; Alonso, Marciani et al. 2007; Eldeghaidy, Marciani et al. 2010) and have found integrations between cortical areas.

1.4.1 Investigating multimodal flavour perception

Interactions between chemical components in a beer could alter the partitioning of volatiles from the aqueous phase to the gaseous phase. Consequently this could impact on delivery to the olfactory bulb and the perception of odour quality and intensity. The relationship between the volatile concentration in the gaseous and aqueous phases can be explored by measuring the changes in headspace volatile concentration in a static system. Any changes in the partitioning of aroma volatiles as a result of variation in matrix components can therefore be determined. Headspace measurements are traditionally carried out by collecting a sample of the headspace at equilibrium, commonly by the use of Tenax traps or coated fibres (Solid Phase Micro Extraction, SPME), which extract the analytes from the gas phase. The analytes are then desorped and separated by gas chromatography and the individual molecules ionised and quantified by mass spectrometry. An alternative is to use a soft ionisation technique based on proton transfer such as atmospheric pressure chemical ionisation (APCI) or proton transfer reaction (PTR), followed by mass spectrometry. Taylor and Linforth (2000) developed a novel interface for APCI-MS analysis which allows headspace sampling directly into the mass spectrometer in real-time. Advantages of this method are that it can cope with water and air and can operate at pressures which allow easy and safe sampling of breath (Taylor, Linforth et al. 2000), so it can successfully be used for collecting in-vivo breath samples. The use of these techniques is paramount to gain a full

understanding of chemical interactions between matrix components and volatiles which could alter human flavour perception.

In order to validate instrumental analysis and further understand human perception, sensory evaluation techniques such as quantitative descriptive analysis (QDA)® (Stone and Sidel 2004) and Spectrum™ are commonly used. Sensory panellists are preselected based on their sensory ability and then undergo general training to enhance detection, discrimination and descriptive skills. Product-specific training follows where panellists are exposed to all samples, from which attributes and references (where applicable) are generated. These product-specific attributes are refined to include only objective terms and the perceptual meaning is clearly defined. Assessment protocol is determined and the panel are trained to rate the intensity on an appropriate scale. Panel performance is reviewed and further training given if needed before the final set of data is generated. The use of sensory evaluation in combination with instrumental analysis provide insights into the level at which interactions are taking place. Further analysis of perceptual interactions requires in depth study of the mechanisms responsible at receptor, neural and cortical levels. Animal studies have gone a long way to increase understanding but do not always correlate to human perception. Electrophysiological and neuroimaging techniques are direct and indirect measures of researching brain activity. In Electroencephalography (EEG) and Magneto-encephalography (MEG) the scalp is covered with electrodes which directly measure rapid changes in neuronal activity by recording the electrical (EEG) or magnetic (MEG) activity generated inside the brain. Excellent temporal resolution makes these techniques valuable for studying the timing of brain processes but limited spatial resolution makes

identifying the origin of activity difficult (Huettel, Song et al. 2009). Nuclear imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) can however provide high spatial resolution but at the cost of limited temporal resolution as changes in brain activity are detected over seconds (Huettel, Song et al. 2009). Both techniques are indirect measures of brain activity with PET measuring metabolic activity and fMRI measuring blood flow. PET relies on the injection of a radioactive tracer compound which gives the brain metabolic activity. PET is then able to detect parts of the brain metabolically associated with a given function. However, the radioactive injections are expensive and invasive making PET undesirable for research purposes with healthy subjects. In contrast, fMRI is a non-invasive technique using strong magnetic fields to measure changes in blood oxygenation associated with a certain sensory, motor or cognitive tasks. Spatial resolution is such that the locus of activity can be identified within millimetres of origin (Huettel, Song et al. 2009). Consequently fMRI can be used repeatedly with the same subject to research the effects of multiple stimuli and interactions between stimuli on brain function.

1.5 VARIATION IN ORAL SENSITIVITY

Investigating multimodal flavour perception is further complicated by a variety of population variations in oral sensitivity which can originate from medical, environmental and genetic differences. Ageusia (total loss of taste) due to damage of taste nerves is very rare and in most cases the cause of taste dysfunction is olfactory in nature (Deems, Doty et al. 1991). The reduced ability to smell is known as anosmia, which can be specific to one particular odour, a

temporary disorder or permanent due to olfactory nerve damage from head trauma or brain damage. Congenital anosmia is the genetic inability to smell from birth. Aging usually brings with it some level of olfactory dysfunction altering flavour perception, usually described as a decreased ability to taste. Environmental factors such as medications, changes in hormonal status and exposure can also alter perception (Duffy 2007). Genetic variation in the ability to taste the compound 6-n-propylthiouracil (PROP) splits the population into PROP tasters and PROP non-tasters. Approximately 70% of people are PROP tasters which can be further divided into those who taste PROP intensely (super-tasters) and those who taste it moderately (medium-tasters), leaving the remaining 30% unable to taste the compound (non-tasters). Furthermore, the number of fungiform papillae on the anterior tongue has also been correlated to PROP sensitivity, somatosensation and taste sensitivity (Miller and Reedy 1990; Zuniga, Davis et al. 1993; Duffy and Bartoshuk 2000; Duffy, Peterson et al. 2004). The newly discovered thermal taster status describes an ability to perceive a phantom taste when the tongue is warmed or cooled (Cruz and Green 2000). Those who perceive a taste are called thermal tasters and have been found to have increased oral sensitivity to tastes, flavour, somatosensory stimuli (Green and George 2004; Green, Alvarez-Reeves et al. 2005; Bajec and Pickering 2008; Bajec and Pickering 2010; Pickering, Moyes et al. 2010; Pickering, Bartolini et al. 2010). Variation in oral sensitivity has also been associated with preference for high-fat foods (Duffy, Bartoshuk et al. 1996; Duffy and Bartoshuk 2000; Thomassen, Faraday et al. 2005), vegetables (Dinehart, Hayes et al. 2006; Bajec and Pickering 2010) and alcoholic beverages (Intranuovo and Powers 1997; Duffy, Davidson et al. 2004; Duffy,

Peterson et al. 2004) which may influence food and beverage intake.

1.6 EXPERIMENTAL APPROACH

Interactions between the primary flavour components (sweetness, bitterness, alcohol and carbonation) in beer have not been investigated previously. This research bridges the gap in the literature using a design of experiments approach.

The objectives of this research were to: develop a model system which models the essential characteristics of lager beer but which can be easily manipulated and manufactured for use in laboratory experiments; determine physico-chemical interactions between matrix components which could alter flavour perception; understand the impact and interactions of the varying ingredients on sensory perception; investigate differences in oral sensitivity between population groups; explore the effect of CO_2 on the cortical response to taste.

The first experiment detailed in chapter 3 investigates physico-chemical interactions between matrix components using instrumental measurements. Human sensory assessments are employed in the following chapter to generate an understanding of perceptual interactions (chapter 4). Chapter 5 investigates genetic variation in taste sensitivity and chapter 6 explores cortical activation to oral stimuli and compares activation differences between population groups. For the design of experiments to be successful, strict control of the matrix components within the beer system is needed. Consequently 6 months were spent developing a

realistic but simple model beer system. The development of the system is detailed in the next chapter (2).

Chapter 2

2. DEVELOPMENT OF THE MODEL BEER SYSTEM

2.1. INTRODUCTION

The model beverage developed for this investigation was intended to be recognised by the panel as 'beer' whilst also allowing strict control of each of the components (sweetness, bitterness, alcohol content and carbonation). A model system (rather than brewed beer) was necessary in order to be able to manipulate each element independently for a scientifically controlled approach. Consequently, a model system was created using ingredients which were determined the most appropriate, including; sweetener, bitter hop acids, ethanol, CO_2, water, aroma volatiles, soluble fibre and colouring. An understanding of the contribution of raw materials and the generation of flavours during the brewing process was needed in order to create a realistic model beer system.

The main ingredients and contributors to beer flavour are water, malted barley, yeast and hops (Briggs, Boulton et al. 2004). Alcohol and carbon dioxide are by-products of fermentation and highly important characteristics of beer flavour. However, it is not only the beer's ingredients that give final flavour but also the processing techniques, as a vast majority of flavour compounds are formed by yeast during fermentation. The general brewing process consists of the following steps; malting, kilning, milling, mashing, hop boil, fermentation, maturation and finishing. The flavour of the final product can be manipulated by changes to this process. In particular, time, temperature and pH control all contribute towards producing the correct flavour profile. The following sections review the stages of beer production giving an overview on the main flavours created from each process and the development of the model beer system.

2.1.1 Overview of the brewing process

2.1.1.1 Malts and malting

Many hundreds of potentially flavour active substances are derived from malts or adjuncts (cereals, sugars or flavourings) and include aldehydes, ketones, amines, thiols and other sulphur-containing substances and phenols (Briggs, Boulton et al. 2004). Dimethyl sulphide (DMS) is a characteristic flavour active volatile of lagers, imparting a cooked cabbage aroma. It is produced by the thermal decomposition of S-methyl methionine (SMM) during the germination process which is converted to DMS during light kilning of lager malt (O'Rourke 2002).

2.1.1.2 Milling and mashing

The kilned malt is milled into 'grist' and is intimately mixed with water into the mashing vessel at a controlled rate and temperature allowing the starch to gelatinize. The mash is held for a period of conversion to allow a mixture of enzymes (diastase) to convert the starch and dextrins to soluble sugars and cause partial breakdown of proteins. A sweet wort results, containing mainly carbohydrates and is rich in flavour extracts dissolved from the malt and adjuncts. Other products include non-starch polysaccharides, proteins and polypeptides, which may have positive effects on beer qualities such as increased viscosity and foam stability. After the mashing process is complete, the sweet wort is separated from the spent grains using a mash filter. The wort is run into the kettle where it is boiled with hops.

2.1.1.3 Hops and the hop boil

Historically hops were added to preserve the beer during fermentation. However, in modern day processing their main

function is to provide flavour. Bitter taste is one of the most important flavours in beer (Meilgaard, Dalgliesh et al. 1979), derived from the addition of hops during boiling or the addition of hop extracts to the wort or even the final beer. Hop resins provide bitterness and essential oils provide aroma which can be flowery, citrus, fruity or herbal, depending upon the variety (Briggs, Boulton et al. 2004). Hops are added to the sweet wort and boiled for 1-2 hours, which isomerizes the α-acids in the hop resins to bitter iso-α-acids (Briggs, Boulton et al. 2004). The degree of bitterness imparted by the hops depends on the degree to which the α-acids are isomerised during the boil and can be estimated by the light absorbance of a solvent extract. The European Brewing Congress (EBC) Analysis Committee has simplified the calculation and results are reported in Bitterness Units (BU) which has been adopted internationally (IBU).

During isomerisation, an intermolecular rearrangement results in two series of five-membered ring compounds, the trans-iso-α-acids and the cis-iso-α-acids (Briggs, Boulton et al. 2004). Each is a mixture of three compounds (r-groups), isocohumulone, isohumulone and isoadhumulone, the ratios of which (and therefore bitterness) vary according to hop variety. Iso-α-acids are not light stable and form the undesirable highly 'sunstruck' flavoured compound 3-methyl-2-butene-1-thiol over time. One way to avoid the formation of this compound is to use the chemically modified iso-α-acids which are formed by reduction of the iso-α-acids with sodium borohydride to produce rho-(ρ)-iso-α-acids, or reduction of tetrahydroiso-α-acids to produce hexahydroiso-α-acids (Briggs, Boulton et al. 2004). These compounds are light stable and can be added to beer as partial or complete replacement of the native iso-α-acids (O'Rourke 2003). A desirable characteristic of pale lager

beers is generally low hop bitterness with high hop aroma, thus lager brewers tend to use varieties that are traditionally low in bitterness and high in aroma thus containing high hop oil to alpha acid ratio (Briggs, Boulton et al. 2004).

2.1.1.4 Fermentation

The hopped wort is cooled, aerated and pitched with yeast. During fermentation yeast metabolizes the sugary extract in the wort to produce ethanol, carbon dioxide and heat, reducing final gravity. Ethanol is present in all beers and is an important characteristic (Meilgaard, Dalgliesh et al. 1979) contributing to taste (Hellekant, Danilova et al. 1997; Mattes and DiMeglio 2001) and perceived viscosity (Nurgel and Pickering 2005). Carbon dioxide also contributes to taste (Chandrashekar, Yarmolinsky et al. 2009) and is essential to the mouthfeel of beer (Langstaff, Guinard et al. 1991), contributing significantly to overall drinking experience (Guinard, Souchard et al. 1998).

A significant number of flavour compounds are also produced during fermentation which are highly dependent upon the type of yeast strain used, the composition of the wort and the fermentation conditions (Briggs, Boulton et al. 2004). The principal flavour metabolites of yeast fermentation are higher alcohols, aldehydes, organic and fatty acids, esters of alcohols and fatty acids which are formed as by-products of the metabolism of sugars and amino acids (Meilgaard 1975). Esters are the most important group of flavour active compounds in beer (Meilgaard 1975). The most abundant is ethyl acetate, with others in much lower concentrations (Briggs, Boulton et al. 2004). Higher alcohols (such as isoamyl alcohol) also significantly contribute to beer flavour (Meilgaard 1975) and are said to impart a warming character to

beers and intensify the flavour of ethanol (Briggs, Boulton et al. 2004).

2.1.1.5 Maturation and finishing

Once the primary fermentation is complete, the beer must be matured to allow flavour and aroma compounds to be refined and developed. Alterations can also be made to the colour and flavour of the beer if desired. For example, caramel colours are often added to bring the colour up to specification and chemically modified isomerized hop extracts, to alter the bitterness. They can be used to derive as much as 100% of bitterness post fermentation (Briggs, Boulton et al. 2004). Most beers are then chilled, filtered (to remove residual yeast), carbonated and packaged.

2.2 THE MODEL BEER SYSTEM

Various components, such as polydextrose to create a base level of viscosity, aroma compounds and colouring were added at constant levels to create a model system which was reminiscent of beer. Polydextrose is a polysaccharide composed of randomly crossed linked glucose with glycosidic bonds and has very low sweetness. When dissolved in water it produces a completely clear liquid with no taste associations, at high levels it imparts a slight sweetness. A lager colour was developed by mixing red, yellow and green food colouring. Blending a lager aroma is considered a difficult task and is the job of flavour chemists. After analytical work analysing commercial lager flavours (using gas-chromatography mass-spectrometry), consultation of the literature and many attempts at blending various volatile compounds it was decided to use a blend (created in-house) containing ethyl actete, isoamyl acetate,

phenethyl alcohol, isoamyl alcohol (2-methylbutanol) and dimethyl sulphide. Using the literature as a guideline for concentrations, the volatiles were blended using trial and error method to create a base beer flavour. At every stage these were tasted against benchmark lagers for aroma/flavour comparisons and also at the different levels of variable components (ethanol, dextrose, hop acids and carbonation levels) to get the final dosage levels correct. This was a lengthy process which took approximately four months to complete. The ingredients selected to represent the components under investigation (sweetness, bitterness, alcohol content and carbonation) were carefully chosen as it was important that they elicited the correct flavour profile.

2.2.1 Sweetness

The residual sugars present in beer are a complex mixture of higher dextrins which cannot be metabolised by yeast. Dextrins are a group of low molecular weight carbohydrates produced by the hydrolysis of starch and contribute to the viscosity and consequently the mouthfeel of the beer (Sadosky, Schwarz et al. 2002). Dextrose (MyProtein, Manchester, UK) is 70-80% as sweet as sucrose and was chosen as it provided a similar taste profile to beer according to a small untrained panel (n=6). Dextrose was added up to a maximum of 30g/L (3%) in order to investigate the effects of sweetness and interactions on taste perception .

2.2.2 Bitterness

Reduced isomerized hop extracts were used to create the desired bitterness level as these are used in industry post fermentation to

either create or adjust bitterness. They are produced by liquid CO_2 extraction from hops. A mixture of 4 parts tetrahydroiso-α-acids (Tetrahop®) and 1 part rho-iso-alpha-acids (Redihop®) (Botanix, Kent, UK) were used to create a desirable bitterness profile. The level of bitterness in most commercial beer ranges from 10 to 60 International bitterness units (IBU), with some reported with up to 100 IBU (Briggs, Boulton et al. 2004). As bitterness is a very important characteristic of beer taste and flavour, it was decided that the maximum bitterness level in the model system would be ~80 IBU to incorporate the majority of commercial beers on the market.

2.2.3 Alcohol content

Food grade ethanol (<99%) (VWR International, UK) was sourced. Alcohol levels in beers can contain up to 12.5% ABV (Briggs, Boulton et al. 2004) with the average content of lager approximately 4-5% ABV. The maximum level at which ethanol could be added in this research was decided upon based on levels commonly found in beer and also ethics, for human sensory assessments. The maximum level was set at 4.5%. The risk of alcohol intoxication does not make it experimentally feasible to test higher levels and this would have significantly reduced the sample set allowed per sensory session.

2.2.4 Carbonation Level

The average CO_2 level of standard lager beer is around 2.5 volumes (5g/L) and 3 volumes for bottled lagers (Briggs, Boulton et al. 2004). In order to determine the effect of CO_2 and possible

interactions on flavour perception the CO_2 level was varied at 3 categorical levels; None = 0 volumes, Low = ~2 volumes and High = ~3.6 volumes. 1 volume equates to 1 litre of CO_2 in 1 litre of water. Food grade CO_2 (BOC, UK) was sourced for all experiments. The importance of carbonation method is two-fold. The levels generated must be accurately measured and easily changed or manipulated from sample to sample and the process must be fairly simple as many hundreds of samples were to be produced throughout the course of this investigation. The use of a pressure gauge is the one recommended by home brewing companies, it produces quick results and is relatively easy and cheap to purchase and set up compared to other methods used to measure CO_2 such as the Orbisphere probe (Stavely, Derbyshire), (Barker, Jefferson et al. 1999). This process is also ideal for carbonating batches of samples. Accurate maintenance of CO_2 pressure and therefore CO_2 levels in the samples during batch carbonation is difficult but paramount to the success of this research. Consequently, time and monetary investment was made developing a batch carbonation system described in the following sections. The original system will first be described as this was used for the first experiment detailed in Chapter 3, followed by the development of the new system used for experiments detailed in chapters 4 and 6.

2.2.4.1 The original system

The original laboratory carbonating apparatus is illustrated in **figure 2.1**.

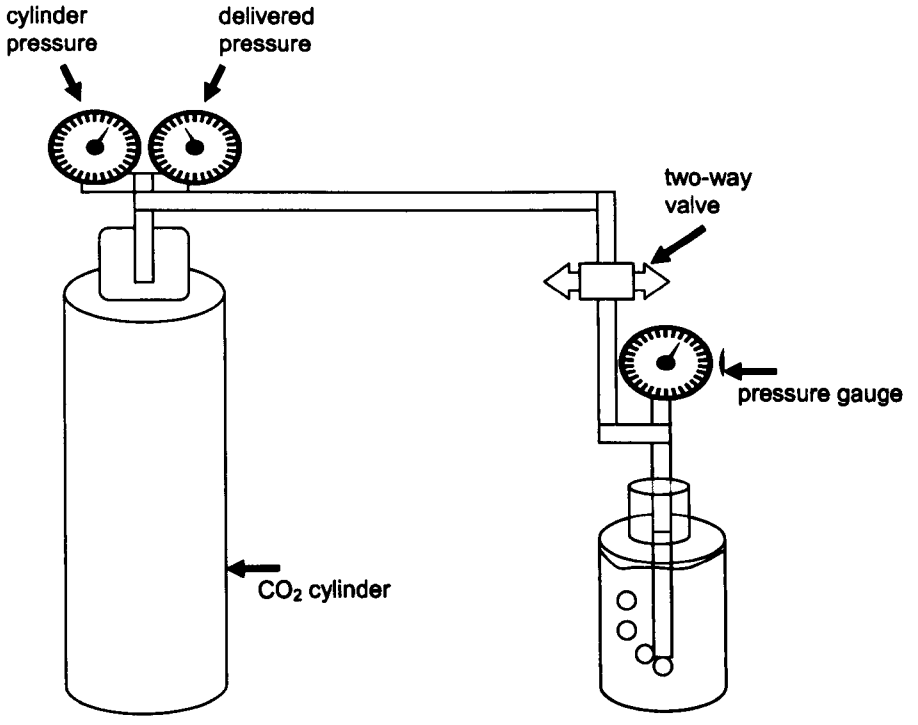

Figure 2.1: Schematic of the original carbonation apparatus

The cylinder of food grade CO_2 (BOC, UK) was connected via a regulator by plastic tubing, flowed by a pressure gauge and more plastic tubing to a bottle lid, allowing the sample bottle to be connected. The flow of CO_2 could be isolated by a one-way valve and therefore allowing the pressure of the sample bottle to be monitored. Once the sample bottle was connected, the pressure was set to the desired level, calculated using the force-carbonation table (**table 2.1**), and the isolation switch opened. The sample bottle was shaken gently to allow full dispersion of CO_2 into the liquid. Once this was achieved, the isolation switch was closed and the pressure within the bottle monitored using the gauge, reopening the isolation switch to top up with CO_2 if necessary. The sample bottle was removed from the apparatus and the sample quickly aliquoted into 40ml glass screw-top vials (Fisher Scientific, Loughborough, UK) and tightly capped to minimise loss of CO_2.

Table 2.1: Force-carbonation table converted to Celsius. Souce: http://sdcollins.home.mindspring.com/ForceCarbonation.html

		GAUGE PRESSURE IN POUNDS PER SQUARE INCH															
		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
	Celsius	VOLUMES of CO_2															
TEMPERATURE	0.0	1.7	1.9	2.2	2.4	2.6	2.9	3.1	3.3	3.5	3.8	4.0	4.2	4.4	4.7	4.9	5.2
	0.6	1.7	1.9	2.1	2.4	2.6	2.8	3.0	3.2	3.5	3.7	3.9	4.1	4.3	4.6	4.8	5.1
	1.1	1.6	1.9	2.1	2.3	2.5	2.7	2.9	3.2	3.4	3.6	3.8	4.1	4.3	4.5	4.7	4.9
	1.7	1.6	1.8	2.0	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.8	4.0	4.2	4.4	4.6	4.8
	2.2	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7
	2.8	1.5	1.7	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
	3.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.7	4.1	4.3	4.5
	3.9	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.0	4.3	4.5
	4.4	1.5	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.3
	5.0	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.2
	5.5	1.4	1.6	1.8	2.0	2.1	2.3	2.5	2.8	2.9	3.1	3.3	3.5	3.6	3.8	3.9	4.2
	6.1	1.4	1.6	1.7	1.9	2.1	2.3	2.5	2.7	2.8	3.0	3.2	3.4	3.6	3.8	3.9	4.1
	6.7	1.3	1.5	1.7	1.9	2.1	2.2	2.4	2.6	2.8	3.0	3.1	3.3	3.5	3.7	3.9	4.0
	7.2	1.3	1.5	1.7	1.8	2.0	2.2	2.4	2.5	2.7	2.9	3.1	3.3	3.4	3.6	3.8	4.0
	7.8	1.3	1.5	1.6	1.8	2.0	2.2	2.3	2.5	2.7	2.8	3.0	3.2	3.4	3.5	3.7	3.9
	8.3	1.3	1.4	1.6	1.8	1.9	2.1	2.3	2.4	2.6	2.8	2.9	3.1	3.3	3.5	3.6	3.8
	8.9	1.2	1.4	1.6	1.7	1.9	2.1	2.2	2.4	2.6	2.7	2.9	3.1	3.2	3.4	3.6	3.7
	9.4	1.2	1.4	1.5	1.7	1.9	2.0	2.2	2.4	2.5	2.7	2.8	3.0	3.2	3.3	3.5	3.7
	10.0	1.2	1.4	1.5	1.7	1.8	2.0	2.2	2.3	2.5	2.6	2.8	2.9	3.1	3.3	3.4	3.6

Although the loss of CO_2 from the liquid was kept to a minimum there was no way of knowing exactly how much CO_2 had escaped and therefore the exact CO_2 level in the samples. Consequently, a new system was designed which allowed the flow of CO_2 directly into the drinking vessel containing the sample which could be isolated and disconnected once the desired CO_2 level had been achieved. The first time the sample would be opened (and gas escape would occur) would be when the panellist themselves were ready to assess the sample. The following section describes the new system which was developed with the aid of an award from DEFRA's Fast Track scheme.

2.2.4.2 The new system

40ml of sample, measured by volume was aliquoted into a 100ml schott bottle (Fisher Scientific, Loughborough, UK). The cap was tightly secured using a silicone sealing ring (RS Components, Corby, UK). A schematic of the batch carbonation system, developed and manufactured in house (Medical Engineering Unit, University of

Nottingham, UK) is detailed in **figure 2.2**. Schott bottle caps (Fisher Scientific, Loughborough, UK) were modified to incorporate a one-way connecting valve (RS Components, Corby, UK) which allows the flow of CO_2 into the sample vessel when connected but is isolated on disconnection. All samples were purged with CO_2 before carbonation commenced. As above, the flow of CO_2 was isolated my means of a shut-off value allowing pressure in the sample bottle to be monitored by a pressure gauge. Samples were carbonated by setting the delivered gas pressure to the desired level, opening the isolation switch and gently shaking the sample bottle to speed the dispersion of CO_2 into the liquid. Once equilibrium was achieved, the shut-off switch was closed to isolate the sample bottle and the pressure within the bottle was monitored using a second pressure gauge to ensure that the correct pressure in the vessel was attained. The sample was disconnected from the carbonation equipment and stored at 5°C (±1) until sampling commenced.

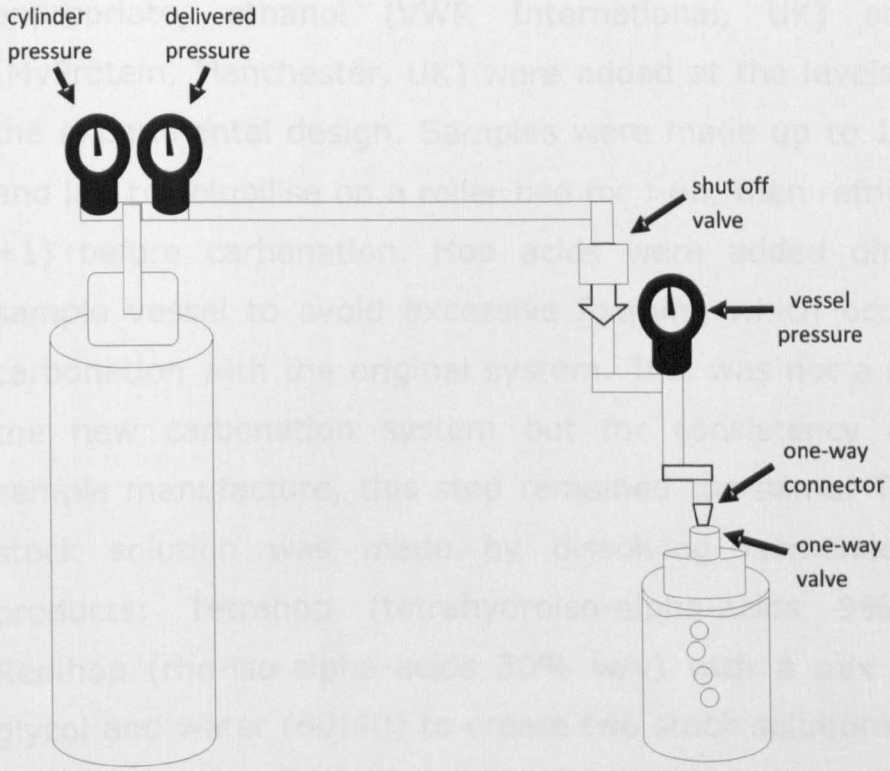

Figure 2.2: Schematic of the new batch carbonation system. The pressure is delivered to the sample vessel via a one-way valve ensuring no gas escape until opening and consumption.

2.3 MODEL BEER MANUFACTURE

Model beer samples were manufactured using 70g/L polydextrose (soluble fibre) (Litesse® Ultra powder, Danisco Sweeteners, KS, USA), water-soluble food colouring (Dr. Oetker, Leeds, UK) comprising of 600μl/L yellow, 50μl/L green and 40μl/L red and a beer flavouring. The beer flavouring was made by dissolving ethyl acetate, isoamyl acetate, dimethyl sulphide, phenethyl alcohol and isoamyl alcohol (2-methylbutanol) in a 60:40 mix of propylene glycol (Fisher Scientific, Loughborough, UK) and Evian water (Danone, France). The beer flavouring was added to obtain final volatile concentrations of; ethyl acetate 3.2μl/L, isoamyl acetate 0.024μl/L, dimethyl sulphide 0.02μl/L, phenethyl alcohol 13.2μl/L and isoamyl alcohol 24μl/L (Sigma Aldrich, Dorset, UK). Where

appropriate, ethanol (VWR International, UK) and dextrose (MyProtein, Manchester, UK) were added at the levels required by the experimental design. Samples were made up to 1L with water and left to solubilise on a roller bed for >6h, then refrigerated (5°C ±1) before carbonation. Hop acids were added directly to the sample vessel to avoid excessive foaming which occurred during carbonation with the original system. This was not a problem with the new carbonation system but for consistency and ease of sample manufacture, this step remained the same. The hop acids stock solution was made by dissolving isomerised hop acid products; Tetrahop (tetrahydroiso-alpha-acids 9% w/w) and Redihop (rho-iso-alpha-acids 30% w/v) with a mix of propylene glycol and water (60:40) to create two stock solutions, (1) 600μl/L and (2) 300μl/L The final hop acid concentration of (1) 600μl/L, comprised of 480μl/L Tetrahop and 120μl/L Redihop (~80 IBU) and (2) 300μl/L, comprised of 240μl/L Tetrahop and 60μl/L Redihop (~40 IBU). For samples containing hop acids, the appropriate concentration of hop acid stock was added directly to empty sample vials (Fisher Scientific, Loughborough, UK). Where the hop acids level was 0 μl/L, an equivalent volume of propylene glycol and water (60:40 mix) was added to ensure sample consistency. All materials were food grade quality.

2.4 EXPERIMENTAL DESIGN

Experimental design software (Design Expert, Stat-Ease Inc, Minneapolis) was used to create a design space varying in the design factors of interest (independent variables); sweetener, hop acids, ethanol and carbonation. The design space is represented schematically in **figure 2.3** to aid visualisation.

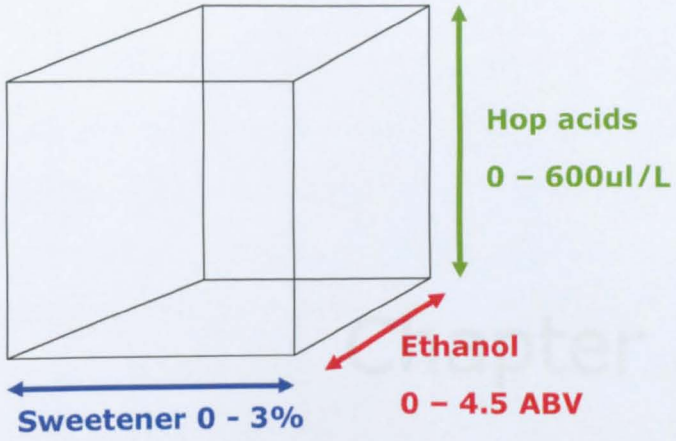

Figure 2.3: Schematic representation of the design space. One design space exists for each level of CO_2 ('none', 'low' and 'high') resulting in 3 design spaces.

The design space allowed the contribution of each independent variable and their interactions on the dependent variables to be assessed. Classical designs, such as full factorial designs with several variable factors create large numbers of samples for testing which is acceptable for instrumental analysis but impractical for sensory assessments. The number of samples for testing can be reduced by reducing the number of design factors and the levels at which they vary or by using a D-optimal design. A D-optimal design can be used with many design factors of which a full range of concentration levels can be investigated. D-optimal designs select a smaller sample number for sensory assessment whilst minimising the variance of the model coefficients. These designs include experimental replicates and are able to produce reliable predictive models of responses using a subset of the total number of potential samples (Eriksson, Johansson et al. 2000). The following chapters will detail the experimental work undertaken and the designs chosen in each.

Chapter 3

3. PHYSICOCHEMICAL INTERACTIONS

3.1 INTRODUCTION

In simple water based solutions, the strength of aroma perception is governed by the volatility of the molecules in the aqueous phase. Compounds partition between air and water depending on their affinity for each phase (Taylor 1998). The partitioning between the aqueous and the gaseous phase is important when investigating aroma delivery to the olfactory bulb via both the ortho-nasal and retro-nasal routes, as changes in partitioning could alter perception and consequently impact upon consumer liking.

Typically, in water based beverages, aroma compounds are present at extremely low concentrations which are considered infinitely dilute and therefore obey Henry's Law. Henry's Law states that "the mass of vapour dissolved in a certain volume of solvent is directly proportional to the partial pressure of the vapour that is in equilibrium with the solution" (Taylor 1998). Therefore, in a closed aqueous system at equilibrium, the concentration of the gaseous phase is directionally proportional to the concentration in the aqueous phase. However, most beverages are not simple aqueous solutions as they contain solutes and other matrix components which could alter the partitioning behaviour of aroma compounds. Furthermore, beverages are not consumed in a closed system at equilibrium and factors such as temperature and dynamic air flow (as opposed to static) may also have an impact.

3.1.1. Instrumental measurement of volatile partitioning

3.1.1.1 Headspace

Classic techniques for analysing flavour volatiles, such as Gas Chromatography-Mass Spectrometry (GC-MS) give detailed structural information but the temporal resolution is not capable of real-time analysis of volatile partitioning (Taylor, Linforth et al. 2000). At the expense of structural information, it is possible to collect real-time data on a mixture of compounds using soft ionisation (providing little fragmentation of ions), followed by mass spectrometry (Taylor, Linforth et al. 2000). Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS) consists simply of an inlet and an ionisation source. The sample is drawn into the heated fused silica capillary inlet. An initial reactant ion is formed from water which transfers its charge to any molecule with a higher proton affinity, such as most organic compounds, at atmospheric pressure. The compounds are ionised by a corona discharge pin and the resultant ions are protonated by the transfer of charge from the reactant ion. Soft ionisation gives sufficient energy to the reagent ions to ionise the molecule, reducing fragmentation which can be controlled by altering the cone voltage. Once formed, the resultant ions are sampled into a standard quadrupole MS under vacuum for quantification. This technique allows controlled sampling of a small amount of equilibrium headspace, therefore reducing disturbance of the equilibrium state. Sampling time is short (commonly <60secs) and takes place in real time. The resultant spectra provide information on the compound's ability to partition from the sample matrix into the gaseous phase (partition coefficient), compared to other matrices. This method can also be used to successfully measure dynamic headspace. An inert gas (nitrogen) dilutes the equilibrium headspace and is sampled into

the APCI-MS to study the stability of volatile headspace concentration in conditions which are closer to those experienced during food and beverage consumption.

3.1.1.2 In-Vivo

Equilibrium conditions are rarely achieved during the consumption of food and beverages. Mastication, swallowing, saliva addition and temperature changes create a dynamic situation (Taylor and Linforth 1996). Liquids are held for a short period in the mouth before swallowing allowing only a short period for aroma release. Swallowing forces the bolus of liquid into the pharynx by the tongue, the velum retracts and elevates preventing the liquid from entering the nasal cavity. After the liquid has passed the epiglottis, the velum is reopened allowing a pulse of aroma into the nasal cavity (Hodgson, Linforth et al. 2003; Hodgson, Langridge et al. 2005; Salles, Chagnon et al. 2011). The highest aroma signal is usually in the first breath after swallowing (Linforth and Taylor 2000). Measuring volatile partitioning in-vivo therefore requires a quick sampling method (such as the APCI-MS) capable of detecting changes in volatile concentration in the breath during consumption (temporal resolution as low as 0.01 sec) whilst not interfering with normal eating patterns. Taylor and Linforth (2000) created a novel interface for the APCI-MS to sample the air from the nose during consumption allowing the study of retro-nasal volatile delivery (Taylor, Linforth et al. 2000). A small disposable plastic tube (10mm diameter and 40-50mm length) is inserted into one nostril so that the assessors could breathe and drink normally with the other end connected to the capillary inlet. The expelled air is sampled and analysed creating traces of breath by breath volatile concentrations. Acetone is a metabolic by-product of blood

oxygenation and is transferred to the breath during exhalation. Consequently, acetone is used as a marker to ensure that the assessors are breathing through their nose during consumption and that the volatiles are entering via the retro-nasal route. This technique has been used to successfully determine volatile partitioning into the breath during mastication and has also been used alongside other techniques to determine the temporal pattern of combined taste and aroma release (Davidson, Linforth et al. 1999).

3.1.2 Effect of ethanol on volatile partitioning

It is important to note that when measuring flavour release from ethanolic samples by APCI-MS, ethanol can interfere with ionisation (Aznar, Tsachaki et al. 2004). However, Aznar (2004) successfully developed a method to control ionisation by adding ethanol to the source to act as the proton transfer reagent ion (Aznar, Tsachaki et al. 2004). Using this method, Aznar (2004) measured the equilibrium headspace of 12% ethanol samples and found that compared to water, 12% ethanol decreased the partitioning of most aroma compounds tested by 4-42% (Aznar, Tsachaki et al. 2004). This is thought to be due to the amphiphilic nature of ethanol, thus decreasing partitioning to the liquid interface for gaseous exchange. Other studies using much higher levels of ethanol have found conflicting results (Conner, Birkmyre et al. 1998; Aprea, Biasioli et al. 2007). Dynamic headspace experiments can be carried out to give a better understanding of the situation which occurs during drinking as the headspace is constantly diluted by gas. Under these conditions there is a reduction in the concentration of volatiles compared to static headspace (Tsachaki,

Linforth et al. 2005). Tsachaki et al (2005) showed that 12% ethanol addition actually boosted the delivery of the majority of volatiles tested to a level which was close to their initial headspace concentration (Tsachaki, Linforth et al. 2005). No literature can be found on the physico-chemical effects of ethanol on volatile partitioning in-vivo and also no current data is available in combination with carbonation.

3.1.3 Effect of carbonation on volatile partitioning

Headspace investigations into the effects of carbonation on volatile partitioning are limited due to the difficult nature of measuring at static equilibrium. Pressurised samples will inevitably become dynamic systems as soon as the sample is opened and the pressure released. This may be the cause of inconsistent results found in other studies (Hewson 2007; Pozo-Bayon, Santos et al. 2009; Saint-Eve, Deleris et al. 2009). Consequently, measures of volatile partitioning from carbonated systems during drinking may provide more meaningful data.

3.1.4 Effect of solutes on volatile partitioning

Volatile partition coefficients could be affected by the solutes in beer such as; inorganic salts, sugars, amino acids, nucleotides, polyphenols and hop resins (Briggs, Boulton et al. 2004) which could physically enhance or decrease solubility of the other compounds within the solution (Taylor and Roberts 2004). No published studies have investigated the effect of hop acids on volatile partitioning. Isomerised hop acids are water soluble up to *circa.* 120mg/l (Briggs, Boulton et al. 2004) and thus unlikely to

alter volatile partitioning on their own in this system. However, they may interact with other components in the matrix as they create foam when in combination with CO_2 which could indirectly alter the surface activity (Briggs, Boulton et al. 2004). Previous research found that sugars, added to water at very high levels (up to 65%) have been found to change the volatility of aroma compounds (Friel, Linforth et al. 2000), which was thought to be due to a phenomenon known as 'salting out' (Voilley, Simatos et al. 1977) rather than a physico-chemical interaction.

Overall, this research project aimed to investigate the multimodal interactions between sweetness, bitterness, alcohol and carbonation on flavour perception. Therefore, it was important to explore if these factors interact at a physico-chemical level within the model system. It is unlikely that the level of sweetener added to the model system in this investigation (3%) would impact due to the very low concentration compared to other studies (Voilley, Simatos et al. 1977; Friel, Linforth et al. 2000) and consequently this was not investigated here. There is limited data on the physico-chemical effects of the individual components (ethanol, carbonation and hop acids) on in-vivo volatile release and they have not been investigated in combination. The aim of this study was to determine the physico-chemical effects of ethanol, carbonation and hop acid addition on volatile partitioning by measuring the volatiles released in the (1) headspace above the samples at equilibrium, (2) headspace above the samples shortly after decanting and (3) exhaled air of people as they consume the beverage.

3.2 MATERIALS AND METHODS

3.2.1 Instrumental measurement of volatile partitioning

A Platform LCZ mass spectrometer, fitted with an MS-Nose interface (Micromass, Manchester, UK), was operated with modifications suitable for use with ethanolic systems. Aznar and co-workers (Aznar, Tsachaki et al. 2004) developed a method which uses ethanol as the charge transfer medium by bubbling nitrogen at different flow rates through a 2% ethanol solution (**figure 3.1**) to achieve a source ethanol content which was constant. If the ionisation environment is kept at 11.3µL ethanol/L N_2, then volatile ionisation is kept independent of sample ethanol content. The release of the three volatiles was measured in selected ion mode, isoamyl alcohol *m/z* 71, ethyl acetate *m/z* 89 and phenethyl alcohol *m/z* 105.

Figure 3.1: Picture of the ethanol set up. The schott bottle contains 2% ethanol solution with nitrogen bubbled through at a flow rate set and maintained by a flow meter.

3.2.2 Effect of ethanol, CO_2 and hop acids on static headspace volatile partitioning

3.2.2.1 Samples

Volatile concentrations for headspace measurements were: ethyl acetate 3.2µl/L, phenethyl alcohol 13.2µl/L and isoamyl alcohol 24µl/L. Isoamyl acetate and dimethyl sulphide were not included as preliminary experiments showed that the concentrations were too low to be detected by the mass spectrometer. Eight samples, varying in ethanol, hop acid and carbonation level at two levels, were prepared using the method detailed in chapter 2 (section 2.3) according a full factorial randomised experimental design as detailed in **table 3.1.**

Table 3.1: Full factorial experimental design detailing the design factors (independent variables) and the levels in investigated

Experimental Design		
Ethanol (%)	Hop acids (µl/L)	CO_2 (volumes)
0	0	0
4.5	0	0
0	600	0
4.5	600	0
0	0	~3.6
4.5	0	~3.6
0	600	~3.6
4.5	600	~3.6

3.2.2.2 Headspace sampling

Static headspace measurements were taken after equilibration and also straight after decanting to mimic volatile delivery similar to a real drinking context. This method is potentially more variable, but may reveal differences in short term release that may not be observed with longer equilibration. For static sampling, aliquots (40ml) of model beer were placed in 100ml Schott bottles (Fisher Scientific, Loughborough, UK) fitted with a one port lid that allowed headspace sampling and were equilibrated at 6°C (± 1) for 2h. Products to be evaluated straight after decanting (short term decanting) were poured from a capped 40ml glass vial directly into a 100ml Schott bottle. A one port lid was fitted and air containing the volatile compounds was sampled into the ionisation source via a silica capillary tube heated to 150°C. Headspace sampling in both cases was at a rate of 5ml/min, for 1min per sample with a dwell time of 0.1s and cone voltage of 15V. Each sample was measured in triplicate following a fully randomised design.

3.2.2.3 Data processing and analysis

The output generated a chromatogram trace of the intensity of the three monitored ions in the headspace during sampling, measured as a percentage of maximum peak height. These values were used in subsequent analysis to show the number of ions formed by ionisation of the sample headspace with a mass to charge ratio of 71, 89 or 105. The average of three replicate peak heights (arbitrary units) for each sample was determined for each compound using MassLynx software (Micromass, Manchester, UK). The mean peak height can then be compared directly between samples to elucidate trends and differences between samples. Analysis of variance (ANOVA) was conducted to identify significant

differences between the samples for each compound measured. Where differences were found predictive polynomial models were generated using Design Expert v6.0.2 (Stat-Ease Inc, Minneapolis) to explain variation in delivery of compounds as a function of ethanol, hop acid and carbonation level. Non-significant terms, as determined by ANOVA were removed to give a mathematical model which best represented the data.

3.2.3 Effect of ethanol, carbonation and hop acids on in-vivo volatile partitioning

3.2.3.1 Samples

Typically in-nose concentrations of volatile compounds are 10 to 100 x lower in than in the breath (Linforth, Martin et al. 2002). Therefore, to increase signal detection, volatile concentrations for in-vivo measurements were increased 2-fold: ethyl acetate 6.4µl/L, phenethyl alcohol 26.4µl/L and isoamyl alcohol 48µl/L. Samples were prepared in the same way as previously detailed in chapter 2, section 2.3. Eight samples, varying in ethanol, hop acid and carbonation level, were prepared according to a full factorial randomised experimental design as detailed in **table 3.1.**

3.2.3.2 In-Vivo Sampling

Panellists opened and immediately consumed approximately two thirds of the 40ml model beer sample directly from the sample vial. A small plastic tube, leading to the MS, was immediately inserted into the left nostril. Once in place, the sample was swallowed and the panellist was instructed to breathe normally through the nose, keeping the mouth closed for the duration of the sampling period. Breath was sampled from the panellist (30ml/min) over a 1min

period after swallowing (dwell time 0.02s, cone voltage 18V). Each sample was consumed in triplicate by 4 panellists using a randomised block design. Each panellist was placed into a separate block to account for individual differences in aroma partitioning caused by differences in physiology and breath flow rates between people (Salles, Chagnon et al. 2011).

3.2.3.3 Data processing and analysis

Mean relative amounts of each compound were determined by comparison of AreaTotal, Area1, Area2, Imax1 and Imax2 parameters (in arbitrary units) which were extracted from chromatograms and integrated using Masslynx software. **Figure 3.2** identifies these parameters on a typical release profile generated from a panellist consuming a sample. The peaks and troughs are the subject breathing out and in respectively. The area of the first peak (Area1) is black and the area of the remaining peaks (Area2) is white. These were combined to give the total area (AreaTotal). The height of the peaks can also be measured, Imax1 is the height of the first peak and Imax2 is the height of the subsequent peak. Volatile air-water partition coefficients (K_{aw} values) were taken from EPI-Suite V4 (Environmental Protection Agency, America).

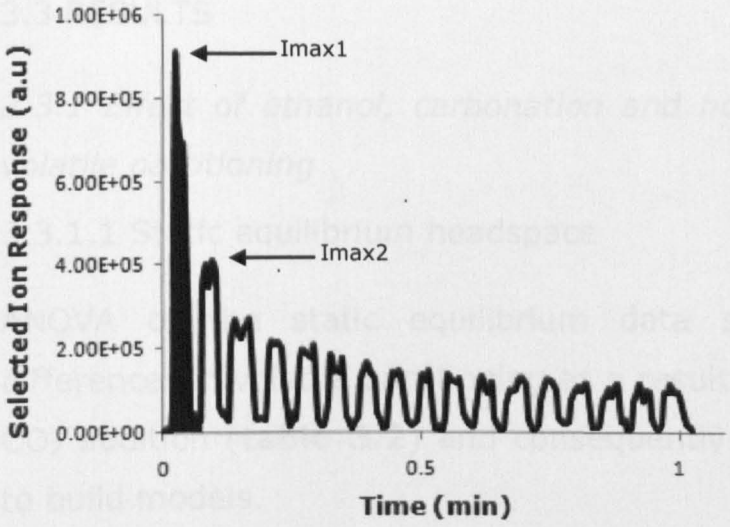

Figure 3.2. Example of a breath by breath release profile collected from 1 panellist after sample consumption. The sample was swallowed at 0 min, volatile delivery was measured for 1 minute afterwards. Imax1 is the height of the 1st peak, Imax2 is the height of the subsequent peak. Area1 is blocked black and Area2 is blocked white. Both black and white areas combine to give AreaTotal.

Analysis of variance (ANOVA) was conducted to identify significant differences between the samples, for each of the compounds measured. Predictive polynomial models were generated using Design Expert v6.0.2 (Stat-Ease Inc, Minneapolis) to explain variations in delivery of compounds as a function of ethanol, hop acid and carbonation level. Non-significant terms, as determined by ANOVA, were removed, and a final mathematical model was chosen which best represented the data after scrutiny of best-fit equations and associated r^2 values. Pearson's correlation coefficient was carried out to determine any significant correlations between parameters.

3.3 RESULTS

3.3.1 Effect of ethanol, carbonation and hop acids on headspace volatile partitioning

3.3.1.1 Static equilibrium headspace

ANOVA of the static equilibrium data showed no significant differences in volatile partitioning as a result of hop acid, ethanol or CO_2 addition (**table 3.2**) and consequently no attempt was made to build models.

Table 3.2: Mean static headspace data in arbitrary units (peak height x 10^4) and standard deviation (SD x 10^4) from 3 replicates of samples varying in ethanol, hop acid and CO_2, after 2h equilibration at 6°C.

Experimental design			Compound (arbitrary units)					
Ethanol (%)	Hop Acids (µl/L)	CO_2 (volumes)	Ethyl Acetate		Isoamyl alcohol		Phenethyl alcohol	
			Mean	SD	Mean	SD	Mean	SD
0	0	0	270	6	81	3	7.2	1.7
4.5	0	0	349	3	100	8	8.1	1.9
0	600	0	306	8	94	30	7.0	2.6
4.5	600	0	299	96	85	31	5.9	2.3
0	0	~ 3.6	314	41	97	16	9.6	2.6
4.5	0	~ 3.6	288	109	84	39	5.7	3.6
0	600	~ 3.6	295	56	95	27	6.9	1.6
4.5	600	~ 3.6	317	81	91	28	8.4	3.1

No significant differences were found therefore no predictive models were built.

3.3.1.2 Short term decanting

This method presents the more dynamic situation of opening a beer and measures the concentration of volatiles which would be delivered orthonasally during this short term event. The model statistics and mean partitioning data for short term decanting are shown in **table 3.3**. Significant effects on ethyl acetate and

isoamyl alcohol partitioning were found but none were found for phenethyl alcohol. An interaction term between hop acids and ethanol ($p<0.05$) showed an increase in the partitioning of ethyl acetate with hop acids but only when ethanol was not present. The partition of isoamyl alcohol into the gas phase was significantly decreased by ethanol addition ($p<0.05$), supporting research by Aznar et al (2004) and Aprea et al (2007). However, as can be seen from **table 3.3**, all mean data is not in agreement with this finding and so interpretation should be treated with caution. Carbonation significantly decreased the partitioning of ethyl acetate ($p<0.0001$) during short term decanting which agrees with the results for other volatiles found by Hewson (2007) using a similar method.

Table 3.3: Mean headspace data in arbitrary units (peak height x 10^4), standard deviation (SD x 10^4) and model statistics from 3 replicates of samples varying in ethanol, hop acid and CO_2, tested immediately after decanting at 6°C.

Experimental design			Compound (arbitrary units)					
Ethanol (%)	Hop Acids (µl/L)	CO_2 (volumes)	Ethyl acetate		Isoamyl alcohol		Phenethyl alcohol	
			Mean	SD	Mean	SD	Mean	SD
0	0	0	307^a	26	139^a	20	6.90^a	2.2
4.5	0	0	298^a	20	108^b	10	5.31^a	1.2
0	600	0	331^b	21	150^a	19	9.89^a	2.8
4.5	600	0	296^a	35	121^b	16	5.90^a	1.5
0	0	~ 3.6	217^c	43	137^a	46	9.25^a	2.7
4.5	0	~ 3.6	250^c	29	126^b	41	6.48^a	3.9
0	600	~ 3.6	282^d	24	133^a	19	9.67^a	2.1
4.5	600	~ 3.6	235^c	21	153^b	16	9.30^a	5.6
Model statistics		R^2	0.67		0.19			
		Adj R^2	0.60		0.15			
		Pred R^2	0.48		0.04			

^{abcd}Samples assigned the same subscript letter within the same column are not significantly different

3.3.2 Effect of ethanol, carbonation and hop acids on in-vivo volatile partitioning

Measuring volatile partitioning in-vivo directly quantifies the volatile delivery experienced during consumption (retronasal release). Drinking is a short-time scale event and the volatiles do not reach an equilibrium state during the process (Linforth, Martin et al. 2002). The addition of ethanol, carbonation and hop acids could change volatile behaviour through differences in surface tension and surface creation not seen in-vitro. These may influence volatile behaviour differently when in contact with the surfaces of the mouth and throat.

Data from the in-vivo breath by breath experiments were expressed as the AreaTotal, which was further divided into Area1 and Area2 (measuring area of the first peak and persistence respectively) and also by height parameters, Imax1 and Imax2 (measuring intensity of first and second peaks), as illustrated in **figure 3.2**. Due to the high level of temporal resolution, Area1 and Area2 can be separated from AreaTotal and quantified, which is important because the two phases result from different processes (Hodgson, Linforth et al. 2003). The volatiles that are released in the first exhalation to form Area1 do so immediately after the sample is swallowed (Hodgson, Linforth et al. 2003). The volatiles released into the breath in subsequent exhalations (Area2) are from a thin layer of residual sample coating the throat during the dynamic gas flow conditions of inhalation and exhalation (Linforth and Taylor 2006). **Tables 3.4 and 3.5** show the mean Area and Imax parameters respectively, and indicate where significant differences occur in a sample. Significant model terms and associated statistics for each compound and parameter are presented in **table 3.6**. The values obtained for Area1 and Imax1

showed a strong positive correlation, with r^2 values greater than >0.93 for all 3 compounds. Consequently, the two measurements would be expected to show similar trends.

Table 3.4: Mean in-vivo Area parameters in arbitrary units (AreaTotal, Area1 and Area2) and standard deviation (SD) from 3 replicates of each sample varying in ethanol, hop acids and carbonation level by 4 panelists, tested at 6°C.

Experimental Design			Area parameters															
Ethanol (%)	Hop acids (µl/L)	CO_2 (vol)	Ethyl acetate						Isoamyl alcohol						Phenethyl alcohol			
			AreaTotal		Area1		Area2		AreaTotal		Area1		Area2		AreaTotal		Area1		Area2	
			Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
0	0	0	2280^{a}	*936*	831^{a}	*392*	1449^{a}	*549*	9062^{a}	*3354*	1791^{a}	*555*	7271^{a}	*2810*	2888^{a}	*1800*	357^{a}	*159*	2531^{a}	*1645*
4.5	0	0	4350^{b}	*1384*	1709^{b}	*637*	2640^{b}	*792*	13626^{b}	*6063*	3257^{b}	*1518*	10368^{b}	*4790*	4847^{b}	*3581*	753^{b}	*546*	4095^{b}	*3036*
0	600	0	2154^{a}	*625*	867^{a}	*368*	1287^{a}	*283*	8238^{a}	*4095*	1943^{a}	*836*	6295^{a}	*3351*	2916^{a}	*2144*	375^{a}	*221*	2542^{a}	*1939*
4.5	600	0	2540^{b}	*1277*	1171^{b}	*526*	2369^{b}	*789*	12684^{b}	*6491*	2944^{b}	*1080*	9741^{b}	*5426*	4153^{b}	*2751*	610^{b}	*330*	3543^{b}	*2424*
0	0	~ 3.6	3151^{c}	*820*	1689^{c}	*639*	1462^{a}	*255*	10391^{c}	*4975*	2988^{c}	*1404*	7403^{a}	*3919*	2998^{a}	*2362*	398^{a}	*258*	2600^{a}	*2129*
4.5	0	~ 3.6	5548^{d}	*1741*	2807^{d}	*1489*	2740^{b}	*344*	14520^{d}	*3775*	4113^{d}	*1462*	10407^{b}	*3265*	4398^{b}	*2029*	631^{b}	*220*	3768^{b}	*1819*
0	600	~ 3.6	3028^{c}	*1057*	1547^{c}	*774*	1481^{a}	*285*	9735^{c}	*3095*	2758^{c}	*674*	6976^{a}	*2745*	2968^{a}	*2102*	423^{a}	*206*	2545^{a}	*1907*
4.5	600	~ 3.6	5393^{d}	*1150*	2570^{d}	*1033*	2824^{b}	*590*	15081^{d}	*6626*	4100^{d}	*627*	10981^{b}	*6106*	4446^{b}	*3015*	715^{b}	*388*	3731^{b}	*2714*

^{abcd}Samples assigned the same subscript letter, in the same column are not significantly different

Table 3.5: Mean in-vivo Imax parameters in arbitrary units (Imax1 and Imax2) and standard deviation (SD) from 3 replicates of each sample varying in ethanol, hop acids and carbonation level by 4 panelists, tested at 6°C.

Experimental Design			Imax parameters									
			Ethyl acetate				Isoamyl alcohol				Phenethyl alcohol			
Ethanol (%)	Hop acids (µl/L)	CO_2 (vol)	Imax1		Imax2		Imax1		Imax2		Imax1		Imax2	
			Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
0	0	0	48.0^{a}	*21.0*	55.0^{a}	*25.0*	82.0^{a}	*22.0*	247.0^{a}	*95.0*	11.0^{a}	*4.4*	91.0^{a}	*52.0*
4.5	0	0	82.0^{b}	*34.0*	102.0^{b}	*36.0*	111.0^{b}	*50.0*	334.0^{b}	*135.0*	23.0^{b}	*14.0*	144.0^{b}	*98.0*
0	600	0	49.0^{a}	*25.0*	45.0^{a}	*11.0*	71.0^{a}	*28.0*	200.0^{a}	*97.0*	12.0^{a}	*7.4*	86.0^{a}	*62.0*
4.5	600	0	67.0^{b}	*45.0*	77.0^{b}	*30.0*	105.0^{b}	*45.0*	308.0^{b}	*165.0*	20.0^{b}	*11.0*	130.0^{b}	*84.0*
0	0	~ 3.6	91.0^{c}	*43.0*	60.0^{c}	*13.0*	107.0^{c}	*63.0*	251.0^{a}	*116.0*	13.5^{a}	*9.8*	93.0^{a}	*70.0*
4.5	0	~ 3.6	140.0^{d}	*66.0*	108.0^{d}	*27.0*	140.0^{d}	*37.0*	359.0^{b}	*90.0*	22.5^{b}	*9.7*	137.0^{b}	*58.0*
0	600	~ 3.6	72.0^{c}	*25.0*	65.0^{c}	*27.0*	88.0^{c}	*28.0*	232.0^{a}	*86.0*	13.0^{a}	*7.6*	93.0^{a}	*65.0*
4.5	600	~ 3.6	111.0^{d}	*26.0*	112.0^{d}	*22.0*	130.0^{d}	*45.0*	378.0^{b}	*212.0*	21.0^{b}	*12.0*	140.0^{b}	*103.0*

^{abcd}Samples assigned the same subscript letter, in the same column are not significantly different

Table 3.6: Significant model terms and associated statistics for each volatile at different parts of the release time course.

Compound	Significant model terms and statistics	AreaTotal	Area1	Area2	Imax1	Imax2
Ethyl acetate	Significant model terms	Ethanol CO_2	Ethanol CO_2	Ethanol	Ethanol CO_2	Ethanol CO_2
	R^2	0.50	0.31	0.57	0.25	0.41
	Adj R^2	0.49	0.30	0.56	0.24	0.40
	Pred R^2	0.44	0.22	0.52	0.15	0.33
Isoamyl alcohol	Significant model terms	Ethanol CO_2	Ethanol CO_2	Ethanol	Ethanol CO_2	Ethanol
	R^2	0.42	0.30	0.38	0.30	0.35
	Adj R^2	0.41	0.28	0.37	0.29	0.33
	Pred R^2	0.34	0.20	0.31	0.21	0.26
Phenethyl alcohol	Significant model terms	Ethanol	Ethanol	Ethanol	Ethanol	Ethanol
	R^2	0.47	0.40	0.44	0.46	0.41
	Adj R^2	0.46	0.39	0.43	0.45	0.40
	Pred R^2	0.41	0.34	0.38	0.40	0.34

The levels of hop acids added to the model beer had no significant impact on volatile delivery in-vivo for any of the measured parameters (**tables 3.4 and 3.5**) and consequently no model was built. Increasing ethanol concentration from 0 to 4.5% was a significant model term for all three volatiles and for all parameters when modelling each volatile's partitioning behaviour (**table 3.6**). Measured as AreaTotal, ethanol significantly increased the in-breath partitioning of ethyl acetate, isoamyl alcohol and phenethyl alcohol ($p<0.0001$) by similar amounts, 43%, 33% and 32% respectively (**figure 3.3**). A significant increase of the Area1 parameter with ethanol addition shows an increased partitioning of all volatiles during the first breath after swallowing ($p<0.001$). This increase was sustained throughout the sampling period as shown by a significant increase of the Area2 parameter ($p<0.0001$).

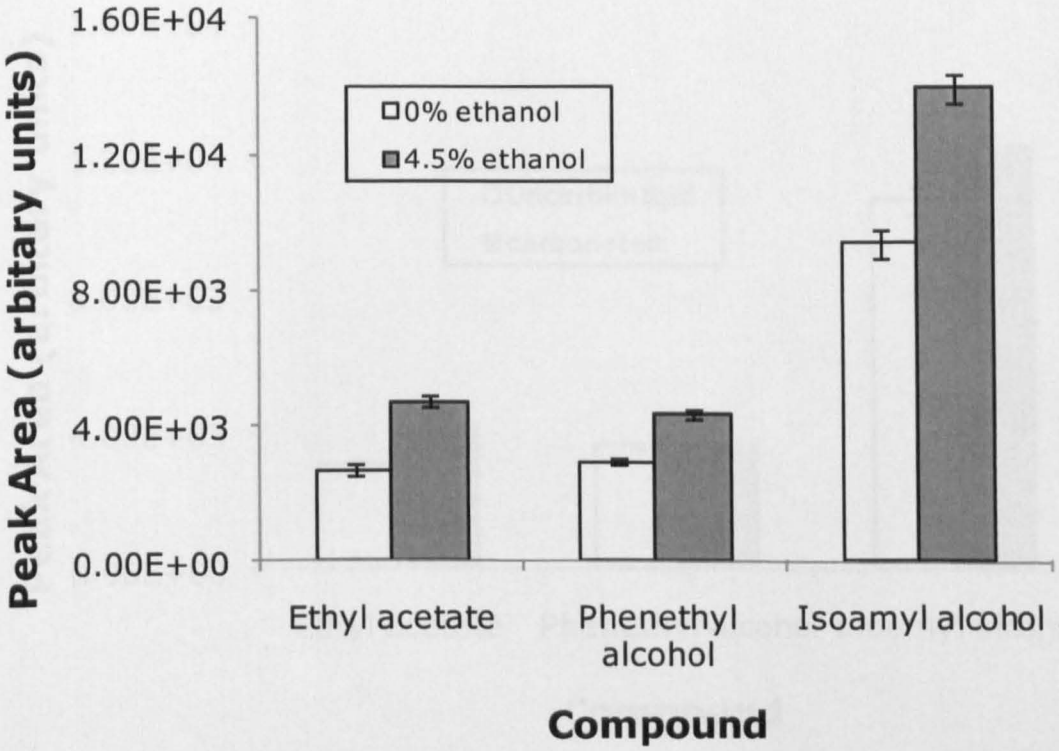

Figure 3.3: Average AreaTotal (arbitrary peak area units) for ethyl acetate, phenethyl alcohol and isoamyl alcohol at 0% and 4.5% ethanol levels when consumed in-vivo (4 panelists, 3 repetitions). The samples were from the experimental design series. Carbonation and hop acids were set to average values. Error bars show the standard error.

ANOVA revealed no significant impact of carbonation for phenethyl alcohol partitioning for any of the parameters measured (**tables 3.4 and 3.5**). Carbonation was a significant model term for ethyl acetate in AreaTotal, Area1, Imax1 and Imax2 parameters and for isoamyl alcohol in AreaTotal, Area1 and Imax1 parameters, as shown in **table 3.6**. Carbonation significantly increased the AreaTotal of ethyl acetate ($p<0.0001$) and isoamyl alcohol ($p<0.0001$), by 28% and 12% respectively (**figure 3.4**).

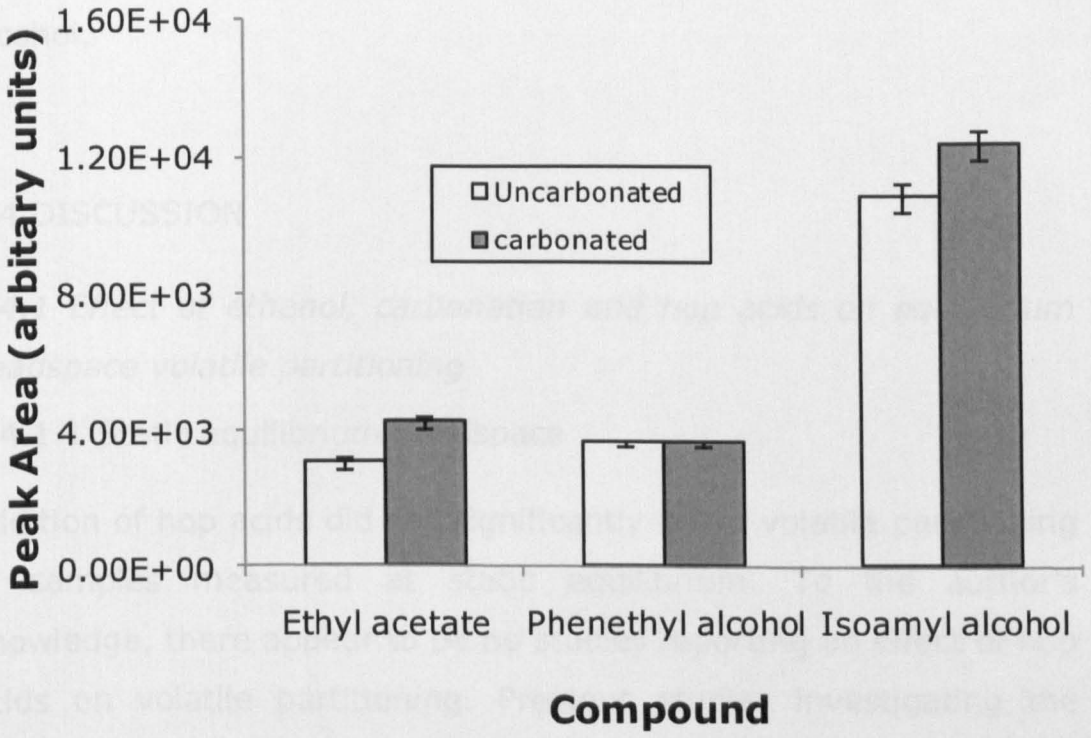

Figure 3.4. Average AreaTotal (arbitrary peak area units) for ethyl acetate, phenethyl alcohol and isoamyl alcohol at uncarbonated (0 vols) and carbonated (~3.6vols) levels when consumed in-vivo (4 panelists, 3 repetitions). The samples were from the experimental design series. Ethanol and hop acids were set to average values. Error bars show the standard error.

Area1 data showed carbonation significantly increased ethyl acetate partitioning by 46%, while isoamyl alcohol was increased by 28%. No significant differences were found in Area2 for any of the compounds indicating that carbonation was not persistent in its effect. The Imax1 parameter shows similar results to Area1 with carbonation significantly increasing ethyl acetate partitioning by 40% and isoamyl alcohol by 20%. Carbonation also significantly increased ethyl acetate ($p<0.01$) partitioning during the 2nd exhalation (Imax2), however this effect was lower in magnitude (19%) compared with Imax1 (40%). For Imax2, the term carbonation was also approaching significance for isoamyl alcohol

(p=0.055), whereas there was no significant effect on phenethyl alcohol.

3.4 DISCUSSION

3.4.1 Effect of ethanol, carbonation and hop acids on equilibrium headspace volatile partitioning

3.4.1.1 Static equilibrium headspace

Addition of hop acids did not significantly affect volatile partitioning in samples measured at static equilibrium. To the author's knowledge, there appear to be no studies reporting an effect of hop acids on volatile partitioning. Previous studies investigating the effect of solutes on volatile headspace concentrations showed that high concentrations are needed to alter volatile partitioning (Voilley, Simatos et al. 1977; Friel, Linforth et al. 2000) and therefore the levels used in this study are unlikely to be sufficient.

Changes in volatile partitioning may have been expected with ethanol addition due to results from previous research. Aznar (2004) investigated the effects of ethanol (12%) on static volatile partitioning compared to water and found a decrease in volatile release due to an increase in solubility of the aroma compounds, reducing their concentration in the headspace by 4-42% (Aznar, Tsachaki et al. 2004). Work by Aprea (2007) also found headspace concentrations of volatiles were reduced when increasing amounts of ethanol (up to 20.9%) were added to the solution (Aprea, Biasioli et al. 2007). Other researchers have found less convincing results with no observed significant differences in activity coefficients of ethyl esters with increasing ethanol concentration (<17%) (Conner, Birkmyre et al. 1998). In light of these previous

studies, a minor decrease in volatile partitioning with ethanol during the static headspace measurements may have been expected in the present study. However, the level of ethanol added (4.5%) was evidently too low to cause a significant decrease.

The addition of carbonation was not found to alter static volatile partitioning as found in other studies (Hewson 2007; Pozo-Bayon, Santos et al. 2009; Saint-Eve, Deleris et al. 2009), however, previous research is limited and results are inconclusive. Saint-Eve et al (2009) reported an increase in headspace volatile partitioning into the gas phase due to carbonation (Saint-Eve, Deleris et al. 2009), while another study found decreases (Hewson 2007) and one found the effects to be compound specific (Pozo-Bayon, Santos et al. 2009). This variation in data may be due to differences in methodology. In this study the headspace was sampled at serving temperature (6°C ±1), whereas others sampled at room temperature which may have affected CO_2 evolution (Liger-Belair, Villaume et al. 2009) and volatile partitioning (Tsachaki, Gady et al. 2008). CO_2 losses are slower over time at lower temperatures (Liger-Belair, Villaume et al. 2009) which may result in a reduced quantity of volatiles being carried away with the escaping gas. In addition, with direct sampling techniques such as APCI-MS and proton transfer reaction-mass spectrometry (PTR-MS), the headspace is often diluted by air as the sample is drawn into the MS. Differences in dilution rates between methods may also affect results. Headspace sampling rates in the present study were equivalent to 8% of the total headspace volume per minute resulting in minimal dilution with time, whereas those carried out by Pozo-Bayon et al (2009) had a headspace sampling rate equivalent to almost two headspace volumes per minute.

Carbonation may have a different effect on volatile behaviour with this level of headspace sweeping.

3.4.1.2 Short term decanting

Hop acids increased ethyl acetate partitioning into the headspace during short term decanting in the absence of ethanol. This could be due to their amphiphilic structure (Briggs, Boulton et al. 2004) which could modify volatile partitioning due to surface tension effects. Ethanol is surface active (Briggs, Boulton et al. 2004) and its addition could have affected the surface tension and/or volatile solubility so that the effect of hop acids is no longer seen. Due to limited research concerning the effects of hop acids on volatile partitioning, interpretation of data requires further study. It should be noted that the predictability of the model (**table 3.3**) is fairly low but this is often the case when the nature of the samples results in considerable variability due to carbonation. Isoamyl alcohol partitioning into the gas phase was significantly decreased by ethanol addition ($p<0.05$). This agrees with previous literature on the effect of ethanol on volatile partitioning as previously discussed (Aznar, Tsachaki et al. 2004; Aprea, Biasioli et al. 2007). The magnitude of this effect was relatively small which may have been due to the level of ethanol addition as this was at a typical beer concentration (4.5%) and consequently lower than those used by other researchers. In addition, interpretation of this result should be treated with caution as the predictive r^2 value of the model is extremely weak (0.04). A significant decrease in the partitioning of ethyl acetate with CO_2 addition was not surprising as this method was designed to capture the effect in the initial pressure change brought about by a carbonated system and agrees with the results found by Hewson (2007) using a similar method.

The initial gas escape could have carried aroma volatiles into the surrounding air at a greater rate than in non-carbonated samples. However this effect was only seen with ethyl acetate and not isoamyl alcohol or phenethyl alcohol.

3.4.2 Effect of ethanol, carbonation and hop acids on in-vivo volatile partitioning

No significant effects of hop acid addition were seen when measured in-vivo. Hop acids are amphiphilic (Briggs, Boulton et al. 2004) and could have affected the formation of the ethanol monolayer affecting surface tension and the Marangoni effect (see below). Despite this, hop acids had no significant effect as a single factor, or in combination with ethanol or carbonation. This is in contrast to the results seen in short term decanting headspace experiments and emphasises the importance of in-vivo measurements.

The significant increases in volatile partitioning into the breath as a result of ethanol addition differ to both the headspace data presented in this paper and work by Aznar (2004). This is not surprising as in-vivo delivery is different to headspace measurements and is similar to those experienced during beverage consumption and hence measured during sensory analysis. This effect may be a result of changes in surface tension, solubility, or the Marangoni effect. Surface tension changes may affect the distribution of the liquid in the mouth and pharynx during consumption (Hodgson, Linforth et al. 2003; Hodgson, Langridge et al. 2005) effectively allowing the sample to spread out and create a larger surface for volatile partitioning. The capacity of ethanol to increase solubility (Aznar, Tsachaki et al. 2004) may help solubilise

the aroma compounds in the aqueous coating of the mouth and throat and prevent losses to other hydrophobic domains. Volatile compounds may also be solubilised at higher concentrations at the aqueous-gaseous interface enhancing release. Ethanol evaporates at the interface transferring volatiles into the gaseous phase. After this initial surface evaporation, ethanol streams to the interface, creating a stirring effect and carrying more volatiles to the surface and via the Marangoni effect (Hosoi and Bush 2001) and this may have caused the increase. A previous study found that under dynamic gas flow conditions, ethanol streaming stirs the sample, increasing volatile partitioning into the gas phase (Tsachaki, Linforth et al. 2005), as found with the present investigation. All of the above effects may alter volatile behaviour resulting in greater delivery into the breath.

The significant increases in volatile partitioning into the breath seem to be limited to the start of the release curve which implies that different mechanisms may be responsible for the more persistent effects seen with ethanol. A study by Saint-Eve et al (2009) investigated the influence of CO_2 on in-vivo aroma partitioning of menthol, menthone and Z-3-hexenol and also split the release profile into separate parameters. When comparing the data presented here and those reported by Saint-Eve et al (2009) several parameters are broadly comparable. In this study, Area2 was the measure of persistence and could be compared to $AUC_{50\text{-}60}$ (area at 50-60 seconds after swallowing), both showing no significant effect of CO_2 on volatile partitioning. Furthermore, data collected immediately after swallowing, Area1 in present study and AUC_2 in Saint-Eve's data set, are broadly comparable measures and show similarities. Comparisons between studies show that menthone (Saint-Eve, Deleris et al. 2009) and ethyl acetate

(present study) partitioning into the breath was increased by a similar magnitude, 50% for menthone and 46% for ethyl acetate. The compounds also have a similar air-water partition coefficient (K_{aw}), menthone ($6.49x10^{-3}$) and ethyl acetate ($4.68x10^{-3}$). Equally, menthol (Saint-Eve, Deleris et al. 2009) and isoamyl alcohol have similar percentage increases (26% and 28% respectively) in delivery due to carbonation and similar K_{aw} values, menthol ($1.05x10^{-3}$) and isoamyl alcohol ($6.70x10^{-4}$). Phenethyl alcohol did not show a significant effect of carbonation and has the lowest K_{aw} ($4.17x10^{-6}$). The effect of carbonation on volatile partitioning into the gas phase appears to follow a trend related to the compound's K_{aw}.

Ethyl acetate has the highest K_{aw}. This could facilitate understanding as to why this compound is significantly more active in its release due to carbonation compared to other compounds investigated in this study. Isoamyl alcohol has a lower K_{aw} than ethyl acetate which could be why the increased effects of carbonation on Imax2 did not quite reach significance. Volatiles with a high K_{aw} become depleted at air-water interfaces during equilibration of liquids with a gas phase. This is because of the proportion of the molecules that have to be transferred (relative to low K_{aw} compounds) to achieve equilibration are larger and is primarily caused by the lack of exchange of molecules between the bulk phase and the interface. The efficiency of delivery can be increased by factors that transfer molecules from the bulk to the interface, which in this case could be CO_2 bubbles.

Ethyl acetate was the only compound to be significantly affected by carbonation in-vitro and in-vivo. However, in-vitro methods showed the opposite effect to those seen in-vivo. Experiments by Pozo-

Bayon et al (2009) using a model mouth found some compounds increased partitioning into the gas phase while some decreased due to carbonation (Pozo-Bayon, Santos et al. 2009). These results represent in-vitro modelling and could be different to actual in-vivo behaviour both in magnitude as well as direction. This illustrates the importance of collecting in-vivo data in order to gain a true representation of interactions influencing volatile partitioning during consumption.

The effect of increased volatile partitioning into the breath by ethanol and carbonation are clearly mediated by different mechanisms. Ethanol remains in the solution and so the increased effect on volatile partitioning is seen throughout the release curve. Whereas CO_2 bubbles disperse and the effect created is only seen at the beginning of the release curve. The cumulative effects of both ethanol and carbonation on volatile release could possibly impact on perceived flavour perception as high partitioning compounds in a mixture could be increased by as much as 86%, while lower partitioning compounds are less significantly enhanced, altering the balance of the flavour as found in other studies (Goldner, Zamora et al. 2009). However, further investigation with more compounds is needed to develop understanding of this effect.

3.5 CONCLUSION

The results of the present study show that hop acid, ethanol and carbonation addition does not affect the static partitioning of volatiles at 6°C. However, it should be noted that the samples used were designed to be representative of beer and higher component levels may have an impact. Short term decanting headspace

measurements did reveal some significant effects of the components. Hop acids interacted with ethanol to increase ethyl acetate release, ethanol decreased isoamyl alcohol partitioning and carbonation decreased the release of ethyl acetate. However these were contrary to those found in-vivo and the models produced were very weak. In-vivo experiments showed that ethanol increased the release of all compounds and carbonation increased the release of high partitioning compounds only, suggesting a relationship between each compound's K_{aw} and their delivery into the breath. The results presented here are of importance to the brewing industry because the combined effects of ethanol and carbonation could increase volatile delivery of high partitioning compounds in the first exhalation after consumption by as much as 86% and thus impact on flavour perception. As results differ between headspace and in-vivo measures, it questions the relevance of relating headspace data to sensory evaluation. Further work is required to compare in-vivo partitioning to human assessments and see if the increases found here are perceivable. The next chapter will investigate the perceptual interactions between the sensory stimuli.

Chapter 4

4. MULTIMODAL INTERACTIONS

4.1 INTRODUCTION

Factors influencing consumer perception are complex and include interactions between flavour components as well as interactions between sensory modalities. There are three levels at which this interaction could occur, (1) physico-chemical interactions occurring in the matrix, (2) interactions at the periphery between one component and the receptors of another and (3) cognitive effects of different stimuli being processed together. Physico-chemical interactions between volatiles and non-volatiles within the matrix may modify perception by altering aroma delivery to the olfactory bulb as investigated in the previous chapter. However, it is currently unknown if these changes are perceivable and what impact this could have on multimodal flavour perception. Flavour is perceived by the detection and integration of stimuli from the gustatory, olfactory and trigeminal systems and interactions between these stimuli can considerably modify sensory perception (Verhagen and Engelen 2006). Beer presents an interesting system to investigate multimodality, as some of the main flavour components are sensed by multiple sensory systems as described in chapter 1.

CO_2 perception is sensed by the trigeminal system but it also stimulates the gustatory system via acid sensing taste receptor cells due to the conversion of CO_2 to carbonic acid (Chandrashekar, Yarmolinsky et al. 2009). Ethanol is perceived by multiple modalities; the gustatory (Wilson, Obrien et al. 1973; Scinska, Koros et al. 2000; Mattes and DiMeglio 2001) olfactory (Laska, Distel et al. 1997; Cometto-Muniz and Abraham 2008) and trigeminal (Green 1991; Trevisani, Smart et al. 2002; Ellingson,

Silbaugh et al. 2009; Goldner, Zamora et al. 2009) systems, although taste seems to be the predominant cue (Mattes and DiMeglio 2001). Sweet and bitter taste is mediated by the G-protein-coupled-receptors (GPCR). The similarities in sweet and bitter transduction has lead to considerable research regarding interactions between the two tastes (Margolskee 2002). Recent research has focussed on specific taste receptor cells (TRC) and their role in sweet and bitter taste interactions indicating peripheral gustatory integration (Talavera, Yasumatsu et al. 2008).

4.1.1 Taste interactions

Taste perception changes when taste stimuli are presented simultaneously and the effect could be that of additivity, enhancement, suppression or possibly even the generation of a new taste. Generally when two tastants are presented together the result is mixture suppression (Pangborn 1960). Research over the years concludes that the outcome of a taste-taste interaction seems to be dependent upon the taste quality, concentration/intensity and temporal aspects (Keast and Breslin 2002). While the detection threshold of a specific tastant in a mixture would seem to increase, thus showing suppression, the overall detection threshold of complex taste mixtures seems to decrease with an increasing number of compounds demonstrating integration (Stevens 1995; Stevens 1997). Integration between tastants in a mixture using concentrations proportional to their separate taste detection level showed that 3, 6, 12 and 24 compound mixtures were detected at concentrations that could not be detected when each compound was presented in isolation. However, while this might suggest simple additivity (complete

addition of the individual components' intensities when rated in isolation), other research would suggest hyperadditivity (the intensity of a mixture is less than the sum of the individual component intensities) (Bartoshuk 1975). In the study by Bartoshuck (1975), subjects were asked to rate the overall perceived intensity of 2, 3 and 4 tastant mixtures and found suppression compared to the simple addition of components when rated in isolation. Therefore, in taste mixtures overall *detection threshold* could decrease even though the individual *compound threshold* could increase.

4.1.1.1 Sweet-bitter interactions

Similarities between sweet and bitter taste transduction has led to considerable research between the two tastes. The addition of a sweetener seems to decrease bitterness perception (Kamen, Kroll et al. 1961; Indow 1969; Bartoshuk 1975; Lawless 1979; Calvino, Garciamedina et al. 1990; Calvino, Garciamedina et al. 1993; Frank, Vanderklaauw et al. 1993; Prescott, Ripandelli et al. 2001) and vice versa (Indow 1969; Bartoshuk 1975; Lawless 1979; Calvino, Garciamedina et al. 1990; Calvino, Garciamedina et al. 1993; Prescott, Ripandelli et al. 2001) Sweet-bitter taste interactions have also been investigated in different matrices and have found similar results (Pangborn, Ough et al. 1964; Calvino, Garciamedina et al. 1990; Calvino, Garciamedina et al. 1993) although the extent of the interaction seems to be dependent upon compound and concentration. Schiffman et al (1994) investigated the effect of various sweeteners on the bitter taste of different bitter compounds at both threshold and suprathreshold. The suppression effect of sweetener addition on detection and recognition thresholds of the bitter compounds was found to be

dependent upon the chemical classification of the sweetener (natural sweeteners were most effective) and the concentration (Schiffman, Gatlin et al. 1994). At suprathreshold levels, sweeteners had a greater effect suppressing bitterness than at threshold (Schiffman, Gatlin et al. 1994).

Work by both Lawless et al (1979) and Prescott et al (2001) determined differences between suppression effects based on PTC/PROP taster status. The suppression of sweetness by PTC (Lawless 1979) and quinine (Prescott, Ripandelli et al. 2001) was found in the taster groups only. As PTC/PROP non-tasters do not have the receptor to detect thiouracil containing compounds, the addition of PTC (Lawless 1979) or quinine hydrochloride (QHCL) (Prescott, Ripandelli et al. 2001) to sucrose did not impact on sweetness perception suggesting peripheral or central interactions. Early work by Kroeze and Bartoshuk (1985) using a split tongue taste paradigm demonstrated that bitterness suppression of quinine by sucrose seemed to be centrally mediated because there was no difference in the level of suppression by sucrose in spatially mixed and the spatially separate 'split tongue' conditions (Kroeze and Bartoshuk 1985). However, more recent work on specific taste receptor cells showed that sweet transduction is directly inhibited by bitter tastants and strongly supports evidence that part of the sweet-bitter interaction is peripherally mediated at the level of the taste receptor cells (Talavera, Yasumatsu et al. 2008).

4.1.1.2 Taste (sweet/bitter)-ethanol interactions

Many alcoholic drinks contain both sweet and bitter taste components, yet the effect of ethanol on taste is unclear. Ethanol has been found to taste predominantly bitter near threshold (Mattes and DiMeglio 2001) and when added at suprathreshold

levels to quinine (Martin and Pangborn 1970; Panovska, Sediva et al. 2008) or to model white wine (Jones, Gawel et al. 2008), bitterness perception was enhanced suggesting an additive effect. However, other psychophysical studies have shown that various levels of ethanol addition increased the sweetness of sucrose (Martin and Pangborn 1970; Panovska, Sediva et al. 2008), while Hoopman et al (1993) found the interaction to be dependent on ethanol concentration. At 10% ethanol, they found increased sweetness intensity and persistence of glucose, sorbitol and xylitol, but the opposite was found at higher ethanol (20-30%) levels (Hoopman, Birch et al. 1993). An investigation into the possible adaptation effects of beer on sweet (12% sucrose) and bitter (0.001 and 0.003% quinine) perception found that both were reduced compared to non-alcoholic beer. Results suggest ethanol may have a suppression effect on both sweetness and bitterness. However, this effect could be ethanol-independent as the alcoholic and non-alcoholic beers were not matched (Mattes and DiMeglio 2001).

Electrophysiological studies have recorded the activation of ethanol from the chorda tympani (Hellekant, Danilova et al. 1997), the glossopharyngeal (Danilova and Hellekant 2000) and lingual branch of the trigeminal nerve (Danilova and Hellekant 2002) in the rhesus monkey, *Macaca mulatta,* in an attempt to uncover the mechanisms behind ethanol taste perception. The chorda tympani nerve (CT) mediates taste from the anterior part of the tongue and therefore the fungiform papillae; while the posterior part is innervated by the glossopharyngeal nerve (GN), providing response from the foliate and circumvallate papillae. The lingual nerve (LN) is part of the trigeminal system innervating the anterior tongue supplying somatic information of oral sensation. CT nerve

responses revealed that ethanol stimulated sweet-best fibres. When 1M (5%) ethanol was mixed with 0.3M sucrose, the stimulation in these sweet-best fibres was significantly increased suggesting ethanol increases the sweetness intensity of sucrose. Furthermore, a cluster of fibres found to respond best to bitter compounds (QHCL and caffeine) did not respond to ethanol. In fact there was a trend that ethanol reduced the response to QHCL in a QHCL/ethanol mixture indicating that ethanol does not taste bitter and actually suppresses bitter taste perception (Hellekant, Danilova et al. 1997). Recordings from bitter-best fibres from the glossopharyngeal nerve in the rhesus monkey support this suppression effect (Danilova and Hellekant 2000).

Evidence from Hellekant et al (1997) that ethanol activates sweet taste receptors is further supported by recordings from gustatory neurons of the nucleus of the solitary tract in rats (Lemon, Brasser et al. 2004). Activation by ethanol was significantly greater in sucrose responsive neurons than unresponsive neurons which increased in a concentration dependant manner and was significantly correlated to sucrose response. Further analysis using multidimensional scaling showed across-neuron patterns of ethanol response to be highly similar to those generated by sweeteners and similarity increased with ethanol concentrations. Furthermore, when a sucrose inhibitor (gurmarin) was applied, both ethanol and sucrose responses were selectively and similarly inhibited, leaving responses to other tastants unaltered providing evidence that ethanol and sucrose stimulate a common gustatory receptor mechanism.

The oral sensation of ethanol was investigated from recordings of the lingual branch of the trigeminal nerve in the rhesus monkey.

The majority (69%) of non-gustatory lingual receptors tested showed increased activity to ethanol suggesting that ethanol elicits a trigeminal response (Danilova and Hellekant 2002). Furthermore, simultaneous ethanol administration with mechanical and temperature evoked stimulation, revealed that the majority of ethanol-responsive fibres were polymodal (also responded to mechanical stimulation and cooling) but were not nociceptive (Danilova and Hellekant 2002). Therefore it seems that ethanol-taste interactions could be complex and involve both gustatory and trigeminal systems.

4.1.1.3 Taste (sweet/bitter)-carbonation interactions

Carbonation has been found to suppress sweetness (McLellan, Barnard et al. 1984; Passe, Horn et al. 1997; Cowart 1998; Odake 2001; Hewson, Hollowood et al. 2009) and increase sourness (Hewson, Hollowood et al. 2009) although data for sweetness suppression is inconsistent (Cometto-Muniz, Garcia-Medina et al. 1987; Yau, McDaniel et al. 1989; Odake 2001; Prescott, Soo et al. 2004; Kappes, Schmidt et al. 2007). The addition of carbonation seems to have no direct effect on bitterness perception in some systems (Cowart 1998; Kappes, Schmidt et al. 2007) but contributes to bitterness or bitter aftertaste in others when no bitter compounds were added in the system (Cometto-Muniz, Garcia-Medina et al. 1987; Cowart 1998; Hewson, Hollowood et al. 2009). The lack of agreement amongst studies varying both in their tastant concentration and CO_2 level indicates that taste-carbonation interactions could be concentration dependent. Furthermore, CO_2 itself seems to act on multiple modalities; oral trigeminal receptors (Simons, Dessirier et al. 1999; Dessirier, Simons et al. 2000; Dessirier, Simons et al. 2001), olfactory

trigeminal receptors (Hu, Zhong et al. 2007) and gustatory receptors (Chandrashekar, Yarmolinsky et al. 2009) which could create complex interactions at a number levels along the gustatory pathway.

4.1.1.4 Taste-aroma interactions

When taste and aroma are perceived together, 'flavour' perception occurs (Taylor and Roberts 2004). The extent to which taste impacts on the aroma and vice-versa has been found to be dependent upon the congruency of the mixture (Frank, Vanderklaauw et al. 1993; Schifferstein 1995; Stevenson, Prescott et al. 1999; Dalton, Doolittle et al. 2000; Hort and Hollowood 2004). Work by Dalton et al (2000) demonstrated that a congruent subthreshold taste (saccharin) and aroma (benzaldehyde) combination could provide an additive effect by significantly reducing the detection threshold of the benzaldehyde when presented simultaneously, which is supported by others (Frank, Vanderklaauw et al. 1993; Schifferstein 1995; Stevenson, Prescott et al. 1999; Dalton, Doolittle et al. 2000; Hort and Hollowood 2004; Pfeiffer, Hollowood et al. 2005). No such effect was found with an incongruent pairing (MSG and benzaldehyde) (Dalton, Doolittle et al. 2000). Results present the possibility of additive integration at a central level which is dependent upon learned experiences. The use of functional magnetic resonance imaging (fMRI) has provided evidence of superadditive responses in cortical regions (anterior cingulate cortex, insula and orbitofrontal cortex) to a congruent bimodal taste and aroma compared to their unimodal entities (Small, Voss et al. 2004). This result was not replicated with an incongruent mixture of NaCl and vanillin (Small, Voss et al. 2004) suggesting that flavour perception is experience-dependent.

Results from both psychophysical and neuroimaging studies strongly indicate that flavour perception is a result of central integration between taste and aroma information in certain cortical regions which is more than the sum of its parts (Small, Jones-Gotman et al. 1997). The extent of this integration would appear to be dependent upon the congruency of the mixture.

4.1.1.5 Carbonation-aroma interactions

There are limited published sensory investigations on the effect of carbonation on aroma perception and conflicting results have been found. Yau (Yau, McDaniel et al. 1989) found that CO_2 significantly increased flavour intensity of a blueberry flavoured milk drink, whereas Lederer (Lederer, Bodyfelt et al. 1991) found that CO_2 suppressed the cooked flavour of milk in flavoured milk products. Both researchers used lower levels of carbonation than is usually found in beer, and milk is a very different system as it includes fat. The melting of fat in the mouth or inversion of emulsion phases can cause substantial changes in volatile partitioning (Taylor and Linforth 1996). Sensory studies on fruit drinks found ratings of fruity apple aroma (McLellan, Barnard et al. 1984) and citrus flavour (Hewson 2007) to be unaffected by carbonation, whereas Kappes et al (2007) found a significant and positive correlation between citrus aroma and carbonation (both sensory attributes). Inconclusive results from these studies may be attributable to the different levels of carbonation used in each study and interactions with other matrix components. Simultaneous presentation of an odour (amyl butyrate) and CO_2 to the nasal cavity has shown suppression effect (Cain and Murphy 1980) suggesting interaction between the olfactory and trigeminal systems. The mechanism for this interaction is unclear and may include peripheral, neural and

central levels dependent upon the odorant, concentration and delivery context (Brand 2006). Interactions could be further complicated by physico-chemical interactions which seem to be compound specific. Instrumental analyses of volatile partitioning from carbonated systems have found conflicting results (Hewson 2007; Pozo-Bayon, Santos et al. 2009; Saint-Eve, Deleris et al. 2009). Findings from the previous chapter (chapter 3) showed that carbonation increased the delivery of high partitioning aroma compounds from a model beer system into the first breath after swallowing but it is currently unknown if this increase is enough to bring about a perceivable difference.

4.1.1.6 Ethanol-aroma interactions

Interactions between ethanol and aroma have been studied at a physico-chemical level where ethanol has been found to increase volatile solubility and thus decrease headspace partitioning (Aznar, Tsachaki et al. 2004) but increase dynamic (Tsachaki, Linforth et al. 2005) and in-vivo delivery (chapter 3). However, it is unknown whether the increases documented in these studies are large enough to be sensorially perceivable. Various aroma attributes of a model white wine containing a reconstructed volatile mixture at 70% to that of original wine concentration were assessed in a study by Jones, Gawel et al. (2008). In samples containing 10g/L glycerol as a sweetener, overall aroma was significantly enhanced when ethanol level was increased from 11% to 13% (Jones, Gawel et al. 2008) indicating that even small increases in ethanol level could impact aroma perception.

4.1.1.7 Ethanol-carbonation interactions

Ethanol and carbonation are often presented together in ciders, beers, wines and alcohol based fruit beverages. There are no studies in the literature investigating the possible interactions between ethanol and carbonation. However, both stimuli have been found to individually alter taste and aroma perception and activate trigeminal pathways, indicating that interactions at receptor and neural levels are possible. It is apparent that there is a lack of knowledge in this area and the current project aims to provide novel research in this domain.

The development of a model beer as discussed in chapter 2, allowed the levels of sweetness, bitterness, alcohol and carbonation to be systematically modified allowing multimodal flavour perception to be investigated. Dextrose, isomerised hop acids, ethanol and CO_2 were included as design factors and the concentration varied within a suitable range for beer. The levels of aroma volatiles, colouring and soluble fibre (polydextrose) were kept constant throughout. This created a model design space from which samples were selected for sensory analysis. The objective of this study was to determine the impact of each design factor on flavour perception.

4.2 MATERIALS AND METHODS

4.2.1 Sensory panel selection

Approval from the University of Nottingham ethics committee was granted before the study commenced. Posters and information sheets with details about the study were displayed in the University of Nottingham Sensory Science Centre to recruit subjects from the

University of Nottingham external sensory panel. Nineteen assessors (3 males) from the external panel volunteered to take part in the screening tests. All assessors had extensive previous experience of sensory evaluation methods with a wide variety of products. Informed consent was obtained from all assessors after the nature of the methods, alcohol content and nutritional consumption per session were fully explained.

Screening tests involved identifying and rating samples varying in ethanol, sweetener, hop acids and CO_2 level for typical attributes in order to establish their ability to discriminate between samples within the model beer design space.

Volunteers were also asked to complete a short alcoholism screening questionnaire (Mayfield, McLeod et al. 1974) which indicates alcohol dependency. The questionnaire asks 4 questions about the volunteer's relationship with alcohol. A positive response to 2 or more of the questions is an indication of alcohol dependency. Any individual scoring 2 or more was not invited to participate in the study and would have been advised to see their General Practitioner.

The volunteers were also screened for their sensitivity to the compound 6-*n*-propylthiouracil (PROP) as this varies genetically (Blakeslee 1932; Glanvill and Kaplan 1965; Bartoshuk, Duffy et al. 1994; Duffy and Bartoshuk 2000; Driscoll, Perez et al. 2006) and has been linked to higher, sensitivity of taste, fat perception, liking/preference of certain foods (Mela 1990; Miller and Reedy 1990; Karrer and Bartoshuk 1991; Duffy and Bartoshuk 2000; Keller, Steinmann et al. 2000; Duffy, Peterson et al. 2004; Lanier, Hayes et al. 2005; Chang, Chung et al. 2006) as well as the number of fungiform papillae present on the tongue (Miller and

Reedy 1990; Duffy and Bartoshuk 2000; Duffy, Peterson et al. 2004). This procedure involves placing a piece of filter paper on the tongue which is supersaturated with 6-n-propylthiouracil (PROP) (Sigma Aldrich, UK). PROP is a medication used to treat Grave's disease (hyperactive thyroid). One paper contains a maximum of 1.2mg of PROP which is less than $1/10^{th}$ of the amount in a single pill. Judges were asked to rate the intensity of bitterness on a general labelled magnitude scale (gLMS) (Bartoshuk et al 1994). Those who rated above moderate were classified to be supertasters, those who rated above barely detectable but below moderate are medium tasters and those who detect very little or who cannot detect anything rated PROP below barely detectable and were classified as non-tasters (Lim, Urban et al. 2008). Fungiform papillae counts were determined using the following method. Commercially available cotton buds (Johnson and Johnson, New Jersey, US) were used to gently apply blue food grade dye (Dr. Oetker, Leeds, UK) to the tongue tip to highlight the amount of taste buds present. Subjects were asked to extend their tongue and a magnifying glass was used to help identify and count the number of fungiform papillae present in a 7mm circle positioned on the tongue. The PROP taster status of each subject was carefully considered before selecting volunteers because of the possibility of sensitivity issues with bitterness perception in the model beer.

19 subjects (3 males) took part in the screening tests. None of the assessors had to be rejected due to failure of the alcoholism questionnaire. 12 subjects scored above 66% on the identification and rating tests were invited to take part, 10 of which agreed. Eight were classified as tasters of PROP and two were classified as super-tasters.

4.2.2 Experimental design space

Experimental design software (Design Expert, Stat-Ease Inc, Minneapolis, MN, USA) was used to create a design space varying in four factors, at 3 levels; ethanol (0, 2.25, 4.5%), sweetener (0, 15, 30g/L), hop acids (0, 300, 600µl/L) and carbonation (none, low and high). For the latter, none corresponded to uncarbonated samples, low to ~2 volumes and high to ~3.6 volumes. A 'volume' is the industry recognised unit of CO_2 measurement and is dependent upon temperature and pressure (Smith and Hui 2004), 1 volume equates to 1 litre of CO_2 in 1 litre of water. Levels were chosen to be perceivably different and representative to levels found in beer.

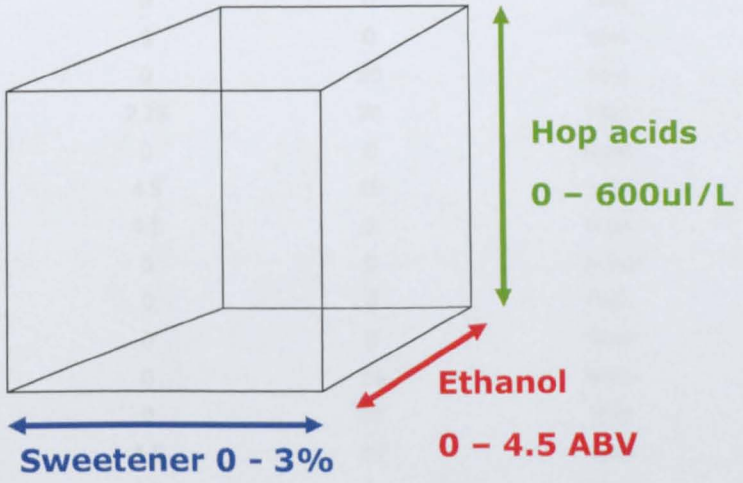

Figure 4.1: Schematic representation of the design space. One design space exists for each level of CO_2 ('none', 'low' and 'high') resulting in 3 design spaces.

Figure 4.1 illustrates the design space and the large quantity of samples which could be generated at each CO_2 level. A D-optimal design was selected to minimise the sample number for sensory assessment whilst maintaining the ability to produce reliable predictive models. The design suggested 31 samples, (including five replicate samples) which are detailed in **table 4.1.** A further

set of 10 samples (validation set) which covered the whole design space were chosen and evaluated in triplicate to allow the predictive models generated by the original data to be validated.

Table 4.1: Samples generated from the D-optimal design

Sample			
HA µl/L	EtOH %	Dex g/L	CO_2
300	4.5	0	Low
0	0	30	None
600	2.25	30	Low
600	2.25	0	High
300	2.25	15	High
300	4.5	30	None
0	2.25	0	Low
300	2.25	15	Low
0	4.5	15	None
0	0	0	Low
600	0	0	Low
0	0	30	Low
0	2.25	30	High
300	0	0	None
600	4.5	15	Low
0	4.5	0	High
0	0	0	None
600	0	0	High
600	0	0	None
600	0	15	None
600	0	30	High
0	4.5	30	Low
0	4.5	0	High
600	4.5	0	None
0	0	0	High
600	0	30	High
600	4.5	0	None
600	2.25	30	Low
0	2.25	30	High
600	4.5	30	High
300	2.25	15	None

4.2.3 Sample preparation and presentation

The preparation of the samples has been described previously in chapter 2, section 2.3. The new carbonation system was used to carbonate the samples, described previously in chapter 2, section 2.2.4.2. Samples (40ml) were served at 5°C (±1) and presented monadically, with 10 minute breaks between each sample. A maximum of 8 samples were evaluated per 2 hour session to ensure no carry over effects or intoxication of alcohol. Each sample was evaluated in triplicate by each panellist over 12 sessions. Samples were presented in a balanced, and randomised presentation order. Unsalted crackers (Rakusens, Leeds, UK), green apple (Asda, Leeds, UK) and Evian mineral water (Danone, Paris, France) were provided for palate cleansing. All tests were performed over a three month period from April – June 2009, at room temperature in an air-conditioned room, under Northern Hemisphere daylight and in individual booths. Data was collected using Fizz software (Biosystems, Cergy-Pontoise, France).

4.2.4 Sensory evaluation

Modified Quantitative Descriptive Analysis (QDA) (Stone, Sidel et al. 1974) was used to profile the sensory attributes of the samples. This method uses the panel's own vocabulary to generate consensus attributes and definitions which were fully discussed to remove any uncertainty of meaning. This method was chosen to be the most appropriate as it benefits from reducing errors associated with 'dumping' of sensations into inappropriate attribute ratings when response alternatives are limited (Clark and Lawless 1994). Samples from the extreme corners of the design space were used

during training to aid with attribute generation, definition, discussion, consolidation, agreement and protocol development. Attribute references were therefore the samples themselves and were used in combination with attribute definitions to standardise language and minimise misunderstanding. Training sessions (40 x 2h) were dedicated to attribute generation, definition, discussion, agreement and protocol development. Rank-rating of selected samples in the sensory booths aided this process. Once the attributes had been consolidated, agreed upon and protocols developed (including order of assessment), practice rating sessions, including replicate data were carried out until the panel could repeatedly quantify between samples for generated attributes. Panel performance during these sessions was monitored by analysing coefficient of variance (CV) between replicates. CV values of less than 25% were considered an acceptable level of variance. Only attributes which the panel agreed upon by consensus and which discriminated between the samples was used. However, cheesy aroma, floral aroma, cheesy flavour and floral flavour were highlighted as difficult attributes during the practice sessions but after discussion with the panel it was decided to keep them in the final attribute list (**table 4.2**). Re-training was given where necessary.

The final set of attributes, their definitions and scale anchors can be found in **table 4.2**. A continuous, unstructured line scale was used to score each attribute. Marks were converted to a score between 0 and 10 for data analysis purposes. All scales were study-specific and labelled with verbal 'anchors' for scale ends which were discussed and agreed upon by the panel.

Table 4.2: The discriminating attributes, descriptions and scale anchors

Attribute	Description	Scale Anchors
Sweaty/cheesy aroma	The sweaty aroma associated with old or blue cheese	Weak - Strong
Floral aroma	A rose-like fragrant aroma	Weak - Strong
Tingly	Painful feeling as bubbles are bursting in the mouth	Not - Very
Carbonation	The presence of bubbles in the mouth	Low - High
Warming	A warm sensation felt all over the mouth after the sample has been swallowed	Not - Very
Astringency	Drying/puckering sensation felt all over the mouth after the sample has been swallowed	Not - Very
Sweetness	Sweetness of the sample whilst held in the mouth	Not - Very
Bitterness	Bitterness of the sample whilst held in the mouth	Not - Very
Complexity of Flavour	The complexity and balance of flavour in the samples	Simple - Complex
Alcohol Flavour	A spicy, whisky-like flavour.	Not - Very
Sweaty/cheesy flavour	The stale slightly acidic flavour associated with old or blue cheese	Low - High
Floral Aroma	A sweet, rose-like flavour	Low – High

4.2.5 Data analysis and panel performance monitoring

Repeatability and discrimination ability of the panel were monitored by assessment of replicate scores. A repeatability index was calculated by FIZZ sensory software (Biosystemes, Cergy-Pontoise, France) using coefficient of variance (CV) subjected to analysis of variance (ANOVA). Two factor (judge, product) analysis of variance (ANOVA) with interaction was conducted for each attribute to

identify significant differences between the samples for each of the attributes assessed. Where appropriate, Tukey's HSD post-hoc tests were used to determine where samples were significantly different (α= 0.05). Predictive polynomial models from panel means were generated using Design Expert to explain variations in perception of each attribute as a function of sweetener, hop acids, ethanol and carbonation levels. Non-significant terms, as determined by ANOVA, were removed. After examination of model statistics, (R^2, adjusted R^2, predicted R^2 and adequate precision), a mathematical model was selected which best represented the data (**table 4.3**). R^2 is a measure of the amount of variation about the mean explained by the model; a value close to 1 shows little variation. The adjusted R^2 (Adj R^2) should be close to the R^2 value to signify that there are only terms in the model which add value. Predicted R^2 (Pred R^2) is calculated by systematically removing each observation from the data-set and estimating the regression equation and determining how well the model predicts the removed observation. This value can range between 0 and 1, with larger values suggesting models of greater predictive ability (Design Expert, Stat-Ease Inc, Minneapolis, MN, USA). Adequate precision (Adeq Precision) measures signal to noise ratio, a value greater than 4 indicates adequate model discrimination (Design Expert, Stat-Ease Inc, Minneapolis, MN, USA). Interaction plots generated by the predictive models were used to visualise key interactions between the design factors. These are not plots of the data points themselves but instead they give a visual representation of the predictive model and are considered more illustrative than the predictive model equations.

The predictive ability of the models was validated by the evaluation of a separate set of 10 samples (validation set) which were taken

from within the design space, representing the full range of compositional factors, but were not part of the original model data set. These predicted values were then compared and plotted against the actual values given by the panel.

4.3 RESULTS

4.3.1 Panel performance monitoring

The repeatability and discrimination ability of the panel was assessed to determine the reliability of the raw data before undergoing full data analysis. Individual panellist performance was assessed by scrutinisation of judge interactions, variation of replicate data and by calculating a repeatability index using coefficient of variance (CV) and the probability value (FPROD) which shows the level of discrimination between products. Results demonstrated that the panel were able to repeatedly discriminate between samples for all attributes ($P<0.05$) except sweaty/cheesy aroma, floral aroma, sweaty/cheesy flavour and floral flavour, as illustrated by **figure 4.2**. Consequently the increases in volatile partitioning caused by ethanol and carbonation as previously found in chapter 3 do not appear to result in perceivable differences in these samples. These non-discriminating attributes were omitted from further study. After examining judge interactions, it became evident that viscosity provided considerable intra-panel variation and consequently could not be used to reliably discriminate between the samples. This is not surprising as it is very difficult for the human palate to significantly discriminate between Newtonian fluids (i.e. lager beer) within such a narrow range (Ragot, Guinard et al. 1989). Furthermore, CO_2 adds a level of complexity to this measurement when it is made in the mouth, increasing turbulence which will impact on shearing stresses and consequently sensory

assessment of beer viscosity (Ragot, Guinard et al. 1989). As a result, the attribute 'viscosity' was not included in further data analysis.

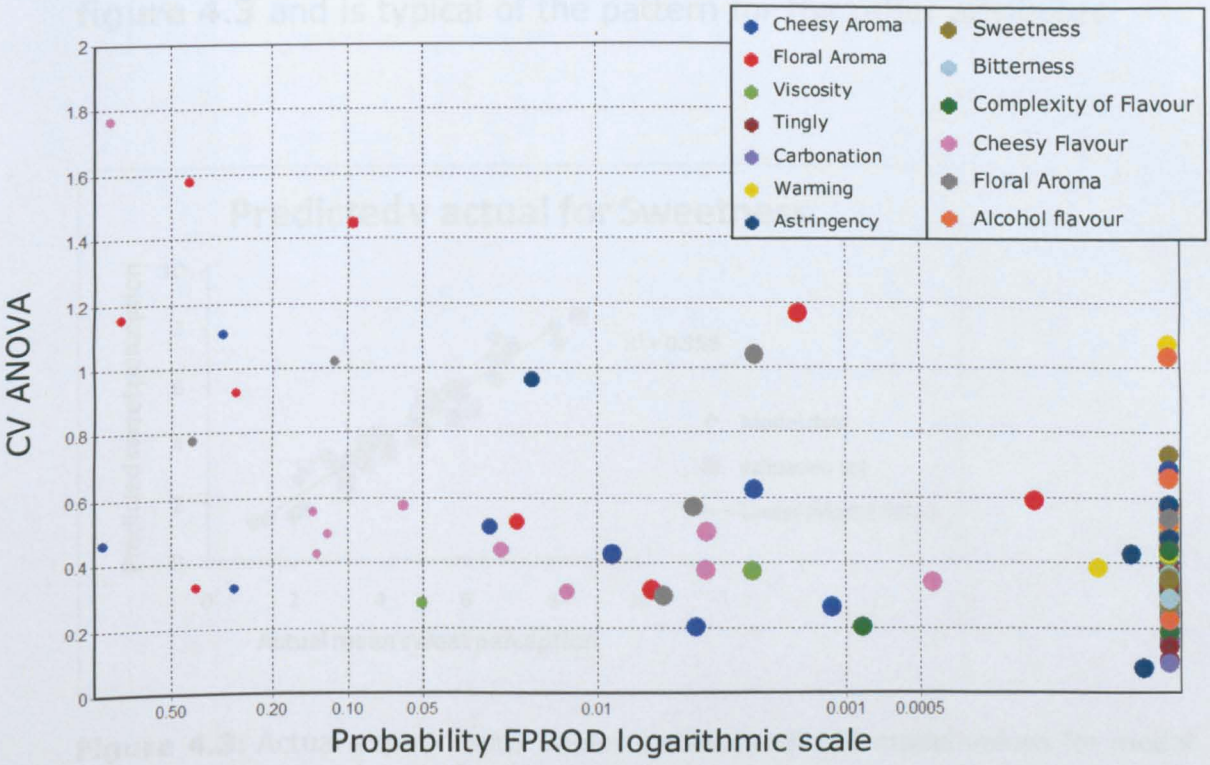

Figure 4.2: Panel monitoring - Repeatability and discrimination. Panel monitoring data for all attributes showing coefficient of variance (CV Anova) plotted against discrimination probability (FPROD). Data points are colour coded for attributes and each data point represents a panellist's mean result for that attribute (3 replicates). The larger the circle, the more significant the discrimination. Ideally all data points should be large and in the bottom right side of the plot.

4.3.2 Model Validation

The independent set of validation samples showed good agreement with model data. Average differences between values predicted by the model and actual values from the validation set for each attribute and across all 10 samples, were <0.6 points on the sensory scale. There was excellent correlation between the experimental mean panel sensory intensity values and predicted

values generated by the models for all attributes (R^2 <0.92). **Figure 4.3** shows an example of this correlation for the sweetness attribute (R^2=0.96). The validation sample set (closed squares) have been overlaid onto the predicted versus actual correlation in **figure 4.3** and is typical of the pattern for the other attributes.

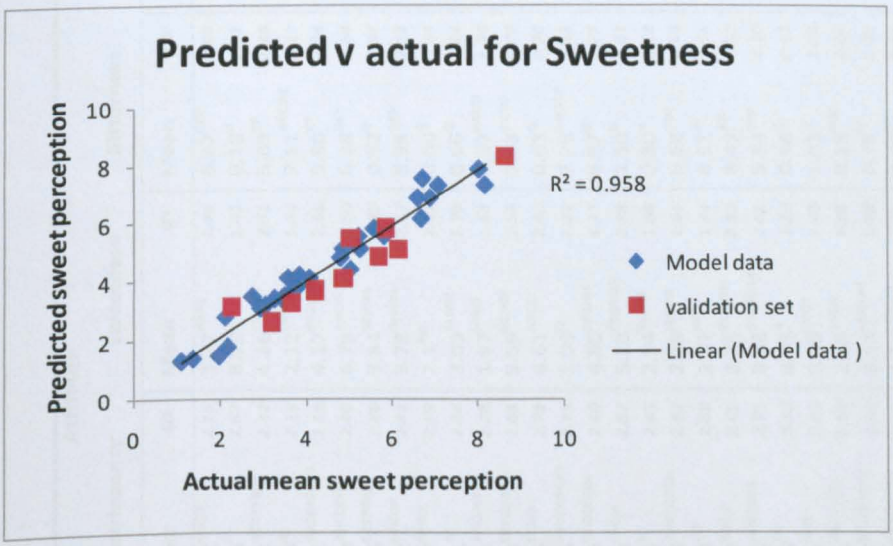

Figure 4.3: Actual experimental values versus predicted model values for model and validation sample sets for the attribute sweetness

Table 4.3: Mean panel scores (from 10 panellists, 3 x replicates each), standard deviation (SD) and post hoc test groupings. HA = hop acids, EtOH = ethanol, Dex = dextrose

Sample				Attribute															
				Tingly		Carbonation		Warming		Astringency		Sweetness		Bitterness		Complexity of flavour		Alcohol flavour	
HA µl/L	EtOH %	Dex g/L	CO_2	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
300	4.5	0	Low	5.40^{CDEF}	2.36	5.12^{CDEF}	2.50	$3.88^{DEFGHIJ}$	2.49	4.82^{ABCD}	2.15	3.27^{JKLM}	2.46	6.87^{CDE}	1.46	5.65^{ABC}	2.19	4.22^{CDEF}	2.75
0	0	30	No	0.08^{G}	0.10	0.06^{G}	0.08	1.76^{MN}	1.92	1.36^{K}	2.67	8.19^{A}	1.41	0.18^{G}	0.31	1.74^{JK}	1.70	1.12^{JKL}	1.59
600	2.25	30	Low	5.29^{CDEF}	2.68	5.24^{CDE}	2.85	$3.85^{DEFGHIJK}$	2.27	$4.02^{BCDEFGHI}$	2.42	4.86^{EFGHIJ}	2.51	5.83^{EF}	2.44	5.38^{ABCD}	2.28	3.42^{DEFGH}	1.95
600	2.25	0	High	5.95^{ABCDE}	2.20	7.00^{AB}	2.43	$2.68^{HIJKLMN}$	1.84	5.78^{A}	2.18	2.12^{LMNO}	1.64	7.11^{ABCDE}	2.21	5.07^{ABCDEF}	2.17	$2.36^{FGHIJKL}$	1.89
300	2.25	15	High	6.49^{ABCD}	2.73	6.65^{ABC}	2.80	$4.26^{CDEFGHI}$	2.58	$4.42^{ABCDEFGHI}$	2.65	$4.17^{EFGHIJK}$	2.66	5.80^{EF}	2.14	5.36^{ABCD}	2.29	3.52^{DEFGH}	2.87
300	4.5	30	No	0.08^{G}	0.12	0.07^{G}	0.12	5.81^{ABC}	2.40	$4.58^{ABCDEFG}$	2.40	6.73^{ABCD}	2.60	6.28^{DEF}	2.64	5.47^{ABCD}	2.49	5.84^{BC}	2.52
0	2.25	0	Low	4.44^{EF}	2.84	4.39^{EF}	2.69	$3.60^{EFGHIJKL}$	2.71	$2.76^{CDEFGHIJ}$	2.88	3.84^{GHIJKL}	1.85	0.92^{G}	1.37	3.16^{GHIJK}	1.86	$2.88^{EFGHIJK}$	3.00
300	2.25	15	Low	5.33^{CDEF}	2.68	4.67^{DEF}	2.69	$3.50^{EFGHIJKLM}$	2.28	4.62^{ABCDE}	2.41	3.78^{GHIJKL}	2.67	5.98^{DEF}	2.13	5.12^{ABCDE}	2.05	$2.79^{EFGHIJK}$	2.41
0	4.5	15	No	0.10^{G}	0.14	0.08^{G}	0.11	7.06^{A}	2.09	2.47^{GHIJK}	2.63	7.1^{AB}	2.51	0.60^{G}	0.94	4.15^{CDEFGH}	2.40	7.93^{A}	2.01
0	0	0	Low	3.78^{F}	2.36	3.65^{F}	2.59	1.86^{LMN}	1.95	1.91^{JK}	2.86	3.05^{KLMN}	1.76	0.66^{G}	1.24	2.15^{IJK}	2.02	0.91^{KL}	1.33
600	0	0	Low	6.27^{ABCD}	2.37	6.23^{ABCD}	2.68	2.23^{JKLMN}	2.09	4.61^{ABCDEF}	2.28	1.97^{MNO}	1.63	7.37^{ABCD}	1.76	4.03^{CDEFGH}	2.58	1.25^{JKL}	1.13
0	0	30	Low	4.49^{EF}	2.67	4.15^{EF}	2.67	$2.81^{HIJKLMN}$	2.29	$2.74^{DEFGHIJ}$	2.66	5.59^{BCDEF}	2.53	0.63^{BCDE}	0.79	3.43^{EFGHIJ}	2.53	1.72^{HIJKL}	2.00
0	2.25	30	High	6.59^{ABC}	2.43	7.28^{A}	2.18	$3.65^{DEFGHIJK}$	2.74	2.65^{FGHIJ}	2.72	6.61^{ABCD}	2.40	0.63^{G}	0.70	3.86^{DEFGHI}	2.61	2.93^{EFGHIJ}	2.92
300	0	0	No	0.09^{G}	0.13	0.08^{G}	0.11	1.77^{MN}	1.39	$4.25^{ABCDEFGHI}$	2.73	1.09^{O}	1.22	7.26^{ABCDE}	2.33	3.31^{FGHIJK}	2.47	0.95^{JKL}	1.08
600	4.5	15	Low	5.36^{CDEF}	2.51	6.00^{ABCD}	2.62	4.92^{BCDE}	2.22	$3.96^{BCDEFGHI}$	2.40	4.80^{EFGHIJ}	3.31	6.97^{G}	2.29	6.17^{A}	2.28	4.74^{BCDE}	2.39
0	4.5	0	High	7.21^{AB}	2.45	7.29^{A}	2.62	4.65^{BCDEFG}	2.98	2.49^{GHIJK}	2.87	5.23^{CDEFGH}	2.38	1.50^{G}	1.63	$4.63^{ABCDEFGH}$	2.62	4.53^{CDEF}	3.42
0	0	0	No	0.10^{G}	0.13	0.08^{G}	0.10	1.19^{N}	1.29	1.45^{K}	2.45	2.94^{KLMN}	1.66	0.30^{G}	0.53	1.65^{K}	1.49	0.67^{L}	0.93
600	0	0	High	7.13^{AB}	2.54	7.50^{A}	2.57	$3.16^{FGHIJKLM}$	2.68	$4.28^{ABCDEFGH}$	2.82	2.18^{LMNO}	1.86	6.68^{CDE}	2.45	$4.61^{ABCDEFGH}$	2.33	1.93^{GHIJKL}	2.17
600	0	0	No	0.09^{G}	0.14	0.10^{G}	0.14	1.24^{N}	1.17	5.28^{AB}	2.63	1.37^{NO}	1.44	8.51^{A}	1.55	3.26^{GHIJK}	2.91	0.90^{KL}	1.01
600	0	15	No	0.09^{G}	0.13	0.07^{G}	0.11	2.12^{KLMN}	1.78	4.99^{ABCD}	2.45	2.75^{KLMNO}	2.53	8.49^{AB}	1.58	3.95^{CDEFGH}	2.35	1.41^{IJKL}	1.59
600	0	30	High	6.59^{ABC}	2.35	7.11^{AB}	2.23	$3.42^{EFGHIJKLM}$	2.44	$4.50^{ABCDEFG}$	2.75	$3.58^{GHIJKLM}$	2.82	5.84^{DEF}	2.10	$4.72^{ABCDEFG}$	2.42	$2.28^{FGHIJKL}$	2.33
0	4.5	30	Low	4.94^{DEF}	2.62	4.91^{DEF}	2.47	6.28^{AB}	2.46	2.30^{IJK}	3.30	8.05^{A}	1.69	0.96^{G}	0.97	$4.87^{ABCDEFG}$	2.67	6.50^{AB}	2.80
0	4.5	0	High	7.48^{A}	2.26	7.21^{A}	2.16	5.37^{ABCD}	2.80	2.41^{HIJK}	3.32	5.83^{BCDE}	2.45	1.01^{G}	1.15	$4.58^{ABCDEFGH}$	2.50	4.96^{BCD}	3.49
600	4.5	0	No	0.09^{G}	0.13	0.10^{G}	0.14	4.57^{BCDEFG}	2.62	$4.51^{ABCDEFG}$	2.34	3.45^{IJKLM}	2.98	8.19^{ABC}	2.09	$4.47^{ABCDEFGH}$	2.47	4.54^{BCDE}	3.08
0	0	0	High	7.12^{AB}	2.40	7.05^{AB}	2.23	2.58^{IJKLMN}	2.45	$3.07^{BCDEFGHIJ}$	3.22	3.51^{HIJKLM}	2.02	0.78^{G}	1.25	2.90^{HIJK}	2.08	1.63^{HIJKL}	1.93
600	0	30	High	6.73^{ABC}	2.52	7.31^{A}	2.59	$3.50^{EFGHIJKLM}$	2.10	$4.30^{ABCDEFGH}$	2.78	4.08^{FGHIJK}	2.86	6.45^{DEF}	2.11	5.44^{ABCD}	2.12	3.38^{DEFGHI}	2.74
600	4.5	0	No	0.12^{G}	0.20	0.13^{G}	0.19	5.34^{ABCDE}	2.34	5.28^{ABC}	2.86	$3.67^{FGHIJKLM}$	2.90	8.26^{ABC}	1.80	5.34^{ABCDE}	2.83	5.53^{BCD}	2.95
600	2.25	30	Low	5.67^{BCDE}	2.66	5.65^{BCDE}	2.99	4.75^{BCDEF}	2.34	$4.33^{ABCDEFGH}$	2.95	5.26^{CDEFG}	2.76	5.96^{DEF}	2.91	5.64^{ABC}	2.31	4.69^{BCDE}	2.41
0	2.25	30	High	7.33^{A}	2.07	7.51^{A}	2.05	4.35^{CDEFGH}	2.67	2.66^{EFGHIJ}	2.97	6.9^{ABC}	2.42	0.93^{G}	1.08	$4.70^{ABCDEFG}$	2.88	3.82^{DEFG}	3.14
600	4.5	30	High	6.28^{ABCD}	2.90	7.23^{A}	2.55	4.97^{BCDE}	2.45	$4.07^{BCDEFGHI}$	2.67	6.69^{ABCD}	2.39	5.01^{F}	2.70	5.99^{AB}	2.65	5.21^{BCD}	2.47
300	2.25	15	No	0.10^{G}	0.15	0.08^{G}	0.13	$2.91^{GHIJKLMN}$	1.42	$3.60^{BCDEFGHI}$	2.44	5.04^{DEFGHI}	2.69	6.19^{DEF}	2.23	$4.27^{BCDEFGH}$	2.20	2.92^{EFGHIJ}	2.19

The panel means, standard deviations and results of Tukey's HSD post-hoc analysis are shown in **table 4.3**. The Tukey's HSD test showed that samples could be split into 7-16 groups (**table 4.3**) indicating a good level of discrimination between the samples across the attributes. ANOVA (judge and product factors) were performed on the panel data (three replicates). Using the global mean of the panellists, polynomial predictive models were generated for each attribute using multiple linear regression (Design Expert, Stat-Ease Inc., Minneapolis). These models described the perceptual results in terms of the design factors (sweetener, hop acids, carbonation and ethanol) for each attribute assessed. The resulting model equations, along with associated statistics describing the model fit (adequate precision) and predictive capability (adjusted and predictive R-squared values) can be found in **table 4.4**.

Table 4.4: Predictive equations generated for design attributes. HA = hop acids, EtOH = ethanol, Adj R2 = Adjusted R^2, Pred R2 = Predicted R^2, Adeq Precision = Adequate precision.

		Significant model terms						Model statistics			
Attribute	CO_2 Level	Intercept	Hop acids	Ethanol	Sweetener	Hop acids^2	HAxEtOH	R^2	Adj R^2	Pred R^2	Adeq Precision
Log10Tingly	no	-1.04	3.31					0.99	0.99	0.99	105.27
	low	0.65	1.80								
	high	0.85	-6.52								
sqrtCarbonation+0.5	no	0.70	2.98E-04					0.99	0.99	0.99	76.05
	low	2.10	5.37E-03								
	high	2.70	-1.14E-04								
Warming	no	1.06	7.55E-03	1.08	0.26		-5.64E-03	0.91	0.89	0.84	19.90
	low	1.92	7.55E-03	0.72	0.26		-5.64E-03				
	high	2.30	7.55E-03	0.58	0.26		-5.64E-03				
sqrtAlcohol Flavour+0.5	no	1.05	2.99E-03	0.34	8.39E-02		-1.50E-03	0.95	0.93	0.90	24.62
	low	1.21	2.99E-03	0.26	8.39E-02		-1.50E-03				
	high	1.37	2.99E-03	0.20	8.39E-02		-1.50E-03				
Sweetness	no	3.07	-0.09	0.45	1.40	1.06E-03		0.95	0.94	0.89	26.70
	low	3.10	-0.09	0.45	0.87	1.06E-03					
	high	3.40	-0.09	0.45	0.78	1.06E-03					
sqrtBitterness+0.5	no	0.94	8.00E-02	4.00E-02	-4.72E-02	-7.62E-04	-1.07E-03	0.99	0.99	0.98	52.70
	low	1.13	7.30E-02	4.00E-02	-4.72E-02	-7.62E-04	-1.07E-03				
	high	1.14	7.10E-02	4.00E-02	-4.72E-02	-7.62E-04	-1.07E-03				
Complexity of flavour	no	1.62	7.00E-02	0.44	0.19	-7.51E-04	-2.34E-03	0.95	0.93	0.90	30.70
	low	2.39	7.00E-02	0.44	0.19	-7.51E-04	-2.34E-03				
	high	2.64	7.00E-02	0.44	0.19	-7.51E-04	-2.34E-03				
Astringency	no	1.68	0.21			-1.10E-03		0.88	0.85	0.80	15.17
	low	2.50	0.09			-1.10E-03					
	high	2.60	0.09			-1.10E-03					

The following sections give the results for each attribute with interaction plots to help visualise the model equations. Some attributes are grouped together in the results and discussion sections because they were significantly correlated.

4.3.3 Tingly and Carbonation

The attribute 'carbonation' relates to the presence of bubbles in the mouth, activated by the mechanoreceptors and was predictably driven by increasing CO_2 level. As indicated in the interaction plot in **figure 4.4**, hop acids interacted with CO_2 to increase perception of carbonation at the low CO_2 level, this effect was not found to be significant at high CO_2 levels. Tingly was used by the panel to describe the painful, chemogenic response from the conversion of carbon dioxide to carbonic acid and as a result, CO_2 was the main driver of tingly perception. As with carbonation perception, hop acids interacted with CO_2 to increase tingly perception at low CO_2 levels. Mean panel results for tingly and carbonation attributes were significantly correlated ($r = 0.99$, $p<0.01$) suggesting that even though the attributes are describing a different action of CO_2 on the trigeminal system, they are unsurprisingly related. This was also found in other studies where attributes such as sting, tingly, fizziness, bubble size and total CO_2 were significantly correlated (Langstaff, Guinard et al. 1991; Hewson, Hollowood et al. 2009).

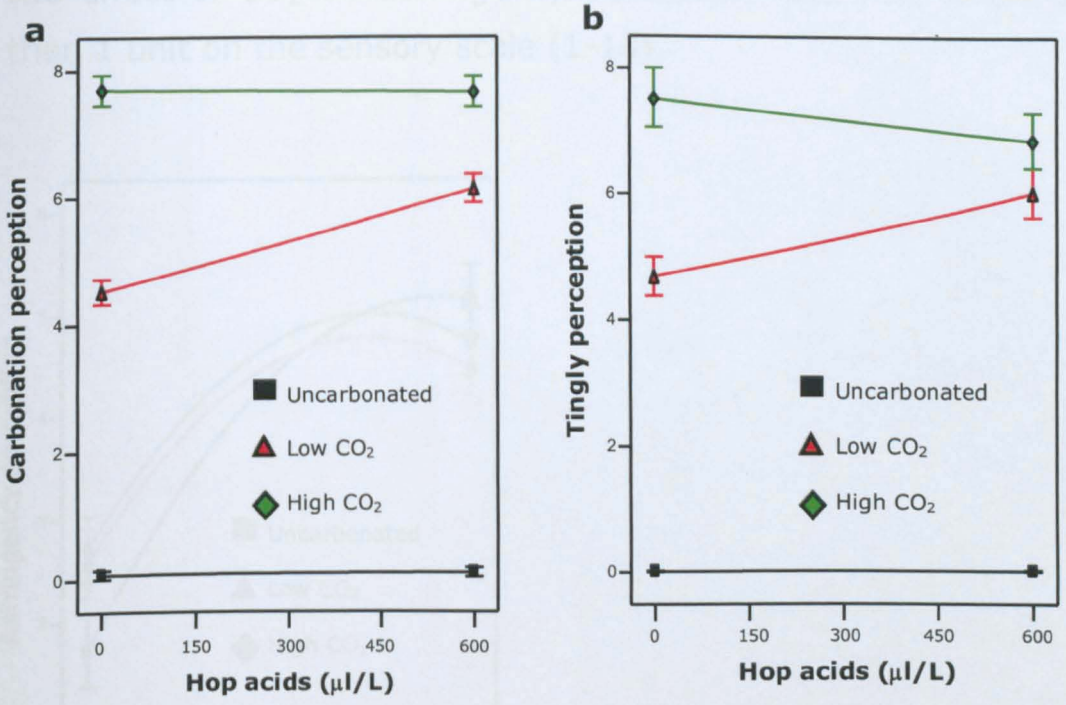

Figure 4.4: Interaction plot generated by the predictive model to illustrate (a) carbonation perception as a function of hop acid and CO_2 addition and (b) tingly perception as a function of hop acid and CO_2 addition

4.3.4 Astringency

Astringency perception was driven by hop acids. However, it is unlikely that the hop acid products used contained any active astringent ingredients, such as polyphenols, as the fractionisation process used to create the hop acids leaves the polyphenols with the spent hops (O'Rourke 2003). It is possible that the panel confused astringency with bitterness or that the two attributes are closely related as suggested by a significant Pearson's correlation coefficient ($r = 0.93$, $p<0.01$). A significant interaction between CO_2 and hop acids existed (**figure 4.5**) and indicates a positive effect of CO_2 on astringency perception at 0µl/L hop acids and a decrease in astringency perception due to CO^2 at 600µl/L. However,

the effect of CO_2 on astringency perception was very small, less than 1 unit on the sensory scale (1-10).

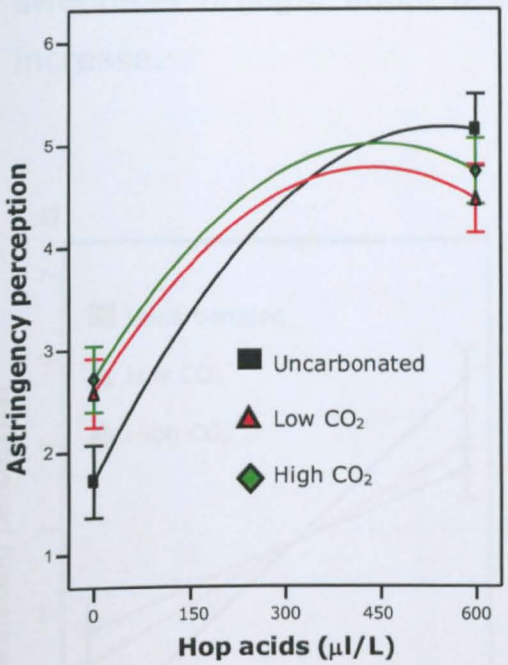

Figure 4.5: Interaction plot generated by the predictive model to illustrate astringency perception as a function of hop acid and CO_2 addition

4.3.5 Warming and alcohol flavour

Warming describes the mouthfeel of ethanol while alcohol flavour was a separate attribute used to discriminate between the samples for the flavour of ethanol. It is likely that the panel were unable to decouple the flavour and trigeminal components of ethanol resulting in significant correlation ($r = 0.97$, $p<0.01$) and generation of similar predictive models. As a result the two attributes will be discussed together here-after. Warming/alcohol perception was primarily driven by ethanol addition in a concentration dependent manner. Hop acids interacted with ethanol to suppress warming perception at 4.5% (ethanol) but contributed slightly when no ethanol was in the system (0%). CO_2

also interacted with ethanol to modify warming perception. At low levels of ethanol, CO_2 contributed to warming perception but detracted from it at high levels (**figure 4.6**). The addition of the sweetener brought about a small, but albeit significant ($p<0.001$) increase.

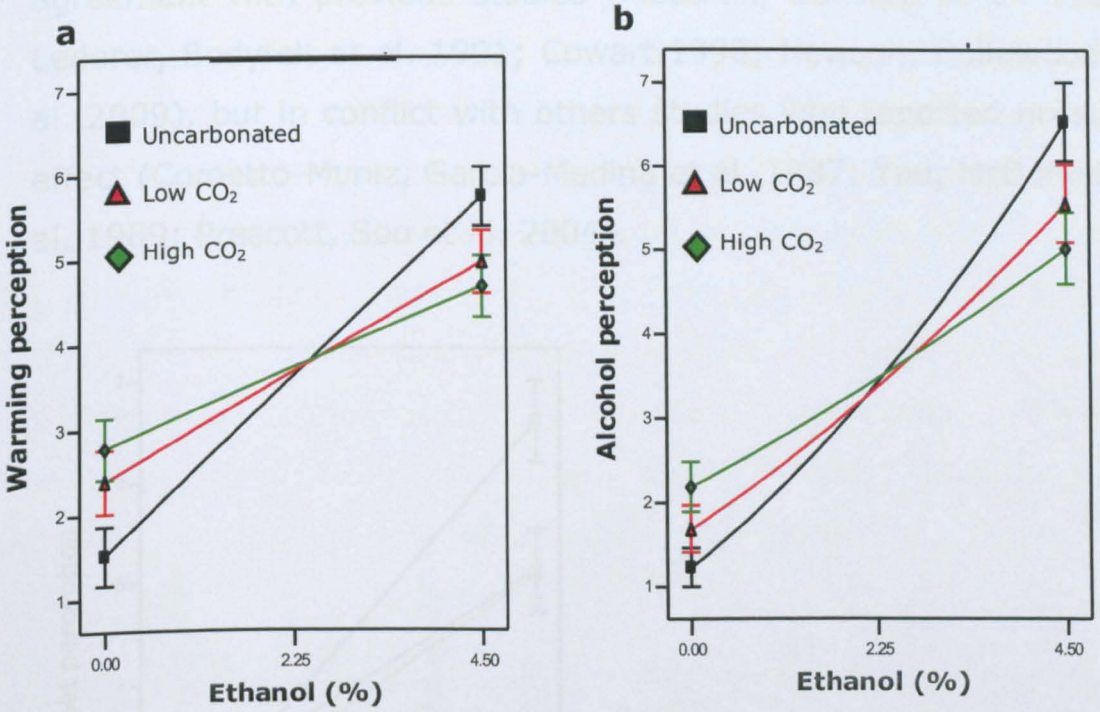

Figure 4.6: Interaction plot generated by the predictive model to illustrate (a) warming perception as a function of ethanol and CO_2 addition and (b) alcohol perception as a function of ethanol and CO_2 addition.

4.3.6 Sweetness

Not surprisingly sweetness was driven by the sweetener, increasing linearly with sweetener concentration. Ethanol also linearly increased sweetness perception with the greatest effect at 4.5% ethanol. This result supports other work on a range of different sweeteners which found an increase in sweetness perception with 10% ethanol addition (Hoopman, Birch et al. 1993) and also with sucrose at ethanol concentrations of 4, 8, 12 and 24% (Martin and

Pangborn 1970) and presumably relates to the gustatory response of ethanol (Hellekant, Danilova et al. 1997). Hop acids significantly suppressed sweetness perception exponentially with the decrease reaching a plateau at approximately 300µl/L hop acids. An interesting interaction with CO_2 reveals that carbonation significantly reduced sweetness perception (**figure 4.7**) which is in agreement with previous studies (McLellan, Barnard et al. 1984; Lederer, Bodyfelt et al. 1991; Cowart 1998; Hewson, Hollowood et al. 2009), but in conflict with others studies who reported no such effect (Cometto-Muniz, Garcia-Medina et al. 1987; Yau, McDaniel et al. 1989; Prescott, Soo et al. 2004).

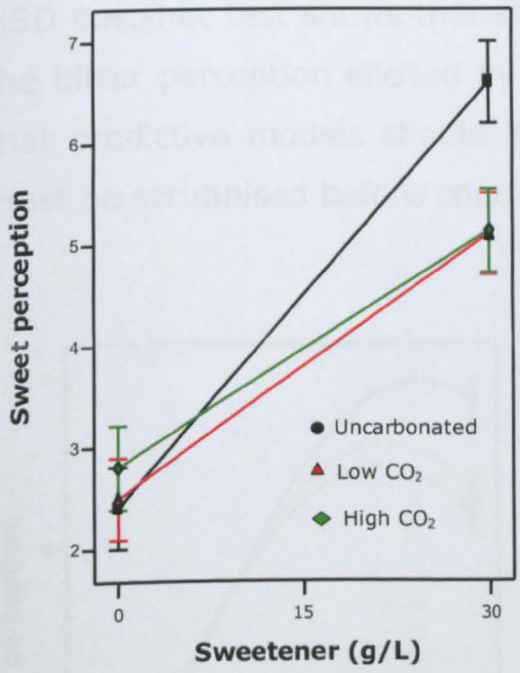

Figure 4.7: Interaction plot generated by the predictive model to illustrate sweet perception as a function of sweetener and CO_2 addition

4.3.7 Bitterness

Bitterness was driven by hop acids as expected, while addition of the sweetener reduced bitter perception which is likely to be due to

mixture suppression (Walters 1996). CO_2 interacted with hop acids to significantly reduce bitter perception at the high levels but contribute at low levels (up to approx. 150ul/L or ~20 IBU) (**figure 4.8**). The 'double opposite' effect of CO_2 on bitterness perception has been previously found with quinine sulphate (Cometto-Muniz, Garcia-Medina et al. 1987). While the contributory effect of CO_2 on bitterness perception was relatively small, it is of significance to brewers as the bitterness level of most lagers falls within this range. The suppression effect began at approximately 300µl/L and was most significant between 450-600µl/L. Ethanol was a significant model term which interacted with hop acids in the predictive model. However, closer examination of the raw data, ANOVA and Tukey's HSD post-hoc test shows that ethanol does not significantly modify the bitter perception elicited by hop acids. This highlights the fact that predictive models should be used with caution and raw data must be scrutinised before conclusions are drawn.

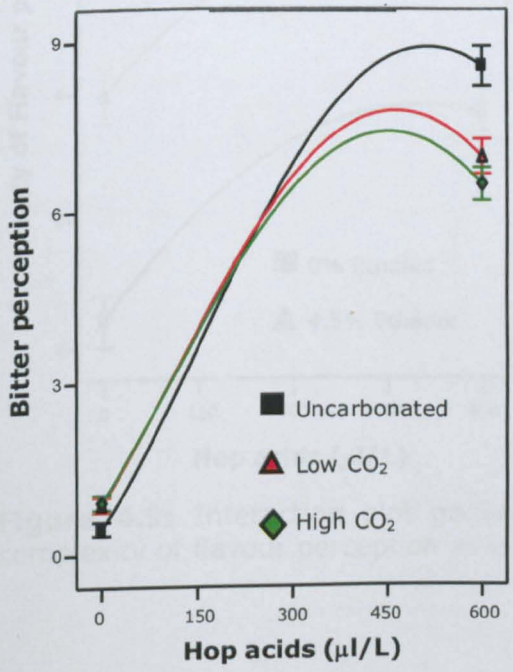

Figure 4.8: Interaction plot generated by the predictive model to illustrate bitter perception as a function of hop acid and CO_2 addition

4.3.8 Complexity of flavour

Complexity is a term which is commonly used to describe wine (Meillon, Viala et al. 2010). In this study it was an all encompassing term used to describe the balance of flavour and mouthfeel attributes. Complexity of flavour was the only attribute which was significantly increased by all design factors. Ethanol was the main driver, followed by hop acids, carbonation, and to a lesser extent, the sweetener. An interaction between ethanol and hop acids (**figure 4.9**) shows that hop acids have a more pronounced effect on complexity when ethanol is not present. These results indicate the importance of ethanol on perceived complexity in beer which may result from the multiple receptors it stimulates and the associated complex transduction pathways.

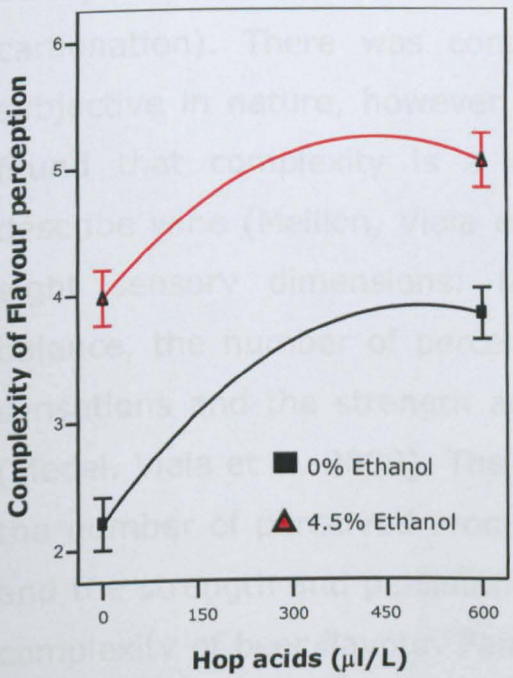

Figure 4.9: Interaction plot generated by the predictive model to illustrate complexity of flavour perception as a function of hop acid and ethanol addition

4.4 DISCUSSION

The attributes generated in this study were similar to those previously used to describe alcoholic and soft beverages (Lyman and Green 1990; Keast and Breslin 2002; Kappes, Schmidt et al. 2006; Bajec and Pickering 2008; Hewson, Hollowood et al. 2009) and some are detailed on the beer and whisky flavour wheels (Meilgaard, Dalgliesh et al. 1979; Shortreed, Rickards et al. 1979). Complexity of flavour was discussed at length by the panel and it was decided that it described the combination and balance of flavour; encompassing tastes, aromas and tactile qualities in the model beer. Samples described as 'more complex' in flavour had more components (sweetness from dextrose, bitterness from hop acids, alcohol flavour/warming from ethanol and tingle from carbonation). There was concern that this term was relatively subjective in nature, however, after study of the literature it was found that complexity is a term which is frequently used to describe wine (Meillon, Viala et al. 2010). It is said to encompass eight sensory dimensions: familiarity, homogeneity, harmony, balance, the number of perceived aromas, the ability to indentify sensations and the strength and persistence of flavour perception (Medel, Viala et al. 2009). The panel agreed that harmony, balance, the number of perceived aromas, the ability to identify sensations and the strength and persistence of flavour were all contributors to complexity of beer flavour. Familiarity was not included as this was thought to bring about a subjective element to the measurement.

4.4.1 Mouthfeel attributes

Figure 4.4a illustrates the interaction between hop acids and CO_2 for carbonation perception. Modelling of tingly data produced a similar interaction plot (**figure 4.4a**). It appears that increasing hop acid concentration is capable of increasing both mechanical and nociceptive response at the low CO_2 level, an effect which is not seen at the high CO_2 level. Informal discussions with the panel revealed that carbonated samples with high hop acid content seemed to have a larger quantity of smaller 'more tingly' bubbles than those without. It is possible that the hop acids interact with the CO_2 to create an increased number of smaller bubbles, increasing surface area and filling the mouth resulting in increased carbonation perception and activating more nociceptors providing an increased tingly response. This seems possible as the isomerised hop acid products used to elicit bitterness could alter surface tension (Briggs, Boulton et al. 2004) and bubble formation. It is likely that this effect is not seen at the high CO_2 level because of CO_2 saturation of both mechanoreceptors and nociceptors, thus any increased effect of the hop acids is not perceivable. With hindsight it would have been beneficial to include bubble size as an attribute in this sensory profile in order to investigate this mechanism further.

Hop acids were the main driver of astringency perception. Considerable time was spent during training to ensure the panel could differentiate between bitterness and astringency. However, astringency has been described as a "complex, multifaceted sensation" by Bajec and Pickering (2008) and its assessment is made difficult by a number of variables. They concluded in their review paper that astringency is sensed by both taste and tactile sensations, suggesting physiological and psychological mechanisms

underly its perception. Polyphenols would usually be the main tactile trigger of astringency in beer, however they were not present in the model beer. Therefore, it is not surprising that in the absence of polyphenols, the bitter taste from the hop acids may contribute to astringency and supports the theory put forward by Bajec and Pickering (2008) that astringency can be sensed by the taste receptors (Bajec and Pickering 2008).

Ethanol stimulates multiple modalities; the gustatory (Hellekant, Danilova et al. 1997; Danilova and Hellekant 2000; Mattes and DiMeglio 2001), olfactory (Laska, Distel et al. 1997) and trigeminal systems (Green 1987; Danilova and Hellekant 2002) resulting in polymodal sensation. Activation of the trigeminal system by ethanol has been found to be (in-part) due to the vanilloid receptor-1 (VR1) (Brasser, Norman et al. 2010) which is the nociceptor responsible for the burning sensation elicited by capsaicin and a wide variety of mechanical, thermal and physical chemical stimuli. It is feasible that this is also the receptor responsible for detecting the warming perception of ethanol. However, the origin of warming perception is currently unknown and could be a result of a non-capsaicin sensitive pathway (Green 1991; Brasser, Norman et al. 2010).

An interaction between ethanol and CO_2 would suggest competition between the trigeminal aspects of both stimuli thus suppressing warming perception (**figure 4.6**). The contributory effect of CO_2 to warming perception at 0% ethanol levels is supportive of this hypothesis. Research focussing on cross-desensitization with capsaicin on ethanol (30%) found a significant decrease in perceived irritation and intensity of 'burning', 'stinging' and 'prickling' sensations of ethanol after treatment of the tongue with

capsaicin (Green 1991) suggesting a suppressive effect when two trigeminal stimuli are presented sequentially. However, further research is needed to determine if this effect is seen when the two stimuli are presented simultaneously.

A minor interaction of hop acids with ethanol suppressed warming perception in a concentration dependent manner with the greatest effect seen at the highest level of hop acid addition eliciting a high level of bitterness to the system. Lim & Green (2007) investigated the relationship between bitter taste and burning sensations and cluster analysis showed that bitterness was more qualitatively similar to 'burning' than any other taste despite being mediated by different sensory modalities. The interaction found in the present study could be due to this similarity.

4.4.2 Complexity of flavour

The significant impact of all four factors on complexity perception illustrates the importance of each in beer flavour perception and also the complex nature of each in a beverage. The ability of ethanol to impact on an array of stimuli and activate multiple modalities means that it is not surprising that it is the main driver of complexity and supports other studies which have also linked increased complexity due to ethanol with liking (Meillon, Viala et al. 2010). Carbonation adds a further level of complexity which is a vital characteristic of beer and has been found to increase thirst-quenching character and drinkability (Guinard, Souchard et al. 1998). Additional work could focus on the contribution of each stimulus on complexity and liking.

4.4.3 Taste attributes

From current knowledge regarding sweet-bitter taste interactions, it is unsurprising that hop acids suppressed sweetness perception. Multiple mechanisms have been proposed for this interaction, with the more recent studies narrowing the locus to the gustatory periphery (Talavera, Yasumatsu et al. 2008) as opposed to higher central neural processing due to similarities between taste transduction mechanisms (Walters 1996; Margolskee 2002). A large body of research exists on bitter-sweet taste interactions with almost exclusive use of quinine as the bitter stimulus. In one such study, quinine was reported to directly inhibit the sweet taste transduction cation channel, TRPM5 (Talavera, Yasumatsu et al. 2008). While sweet perception is not exclusively TRPM5-dependant, this work suggests that other bitter compounds may affect the perception of sweet taste by altering TRPM5 function and significantly suppressing sweet perception, as suggested by the results in the present study. However, research of the pathways responsible for hop acid transduction is required before conclusions about the mechanism of this interaction can be made. Furthermore, the above mechanism does not support the suppression of bitter perception with sweetener addition because bitter signal detection and processing was found to be unaltered by four different sweeteners; sucrose, fructose, saccharin and SC-45647 (Talavera, Yasumatsu et al. 2008). Further investigation into both TRPM5-dependent and independent sweet and bitter transduction pathways are required to determine the source of the interaction.

The additive effect of ethanol to sweetness perception has been previously reported (Martin and Pangborn 1970) and ethanol itself has been described as sweet (Wilson, Obrien et al. 1973). The mechanism for this seems to be a taste-taste interaction as ethanol

has been found to stimulate sweet best fibres in the rhesus monkey (Hellekant, Danilova et al. 1997) and neuronal response to ethanol was similar to that evoked by sucrose in rats (Lemon, 2004). This mechanism has also been extended to humans where ethanol has been suggested to activate some nerve fibres sensitive to sugar (Scinska, Koros et al. 2000) which could explain the source of the interaction as found in this study.

The effect of CO_2 on taste perception is interesting. CO_2 is able to significantly decrease the sweetness of dextrose and modify the bitterness of hop acids. Whether the mechanism of these interactions is peripheral or as a result of higher central processing requires further investigation. However, similarities can be drawn from the study of capsaicin as different classes of oral irritants have been suggested to be mediated partly by a common population of capsaicin sensitive fibres (Carstens, Kuenzler et al. 1998). Physiological interactions between oral irritation by capsaicin and various tastants has been investigated in electrophysiological experiments with rats and also in human sensory studies (Lawless and Stevens 1984; Simons, Boucher et al. 2003). Results found significant suppression of capsaicin on taste which is in agreement with the present study using CO_2. Gustatory neuronal stimulation recordings in the nucleus tractus solitaries (NTS) of rats before and after capsaicin application provide strong evidence that the mechanism of this suppression is peripheral, acting directly on the taste receptor to alter gustatory response (Simons, Boucher et al. 2003). However this does not explain the contributory effect of CO_2 on bitterness perception when no bitterness is in the system or at low levels of hop acid addition. Green & Hayes (2003) investigated the relationship between bitter taste and chemesthesis using capsaicin and suggested two

mechanisms; (1) capsaicin may stimulate the taste neurons which express VR1 and thus stimulate bitterness or (2) capsaicin may stimulate one or more members of the heterogeneous family of T2Rs that encode for bitter taste. Conversely, CO_2 is also mediated by non-capsaicin sensitive pathways (Carstens, Kuenzler et al. 1998) which may interact with gustatory stimuli via centrally mediated integration. The effect of CO_2 on taste perception requires further study as its comprehensive understanding is important for many food and beverage industries.

4.5 CONCLUSIONS AND SUMMARY

The interaction between CO_2 (at low levels) and hop acids on enhancement of carbonation and tingly perception is interesting and requires further study especially at a time when the brewing industry is moving towards reducing CO_2 levels in beers (Bridge 2011). It is possible that with the right combination of ingredients, CO_2 levels could be reduced without significant effect on carbonation and tingly perception. CO_2 interacted with the other variable components in the system (ethanol, sweetener and hop acids) to suppress the perception of warming, sweetness and bitterness attributes respectively at the higher end of component concentration but contributed to perception at the lower end, showing a double and opposite effect. Therefore these results highlight that adjustments and careful consideration would need to be made to the brewing process and ingredients used in order to maintain acceptable levels of sweetness, bitterness and warming perception in low CO_2 beers.

This research has provided understanding into the interactions of ethanol and varying alcohol content on flavour perception. For

example, ethanol was the main driver of flavour complexity which illustrates the importance of its presence in beer. The recent announcement of the 50% reduction on taxation of beers at 2.8% ABV and under (GreatBritian 2011) is likely to increase motivation to develop low alcohol beers. This research also supports the knowledge that ethanol is a complex stimulus acting on different receptors and capable of modifying flavour perception as well as aroma partitioning during consumption.

Hop acids were found to act in a similar way to the much researched quinine on suppression of sweetness perception suggesting that hop acids can also act directly on the gustatory periphery as well as centrally to inhibit the sweet taste transduction pathway. Results support previously inconclusive evidence that CO_2 addition suppresses sweetness perception. The mechanism of this is unknown and could be the result of suppression at the periphery or integration of higher central processing.

Chapter 5

5. TASTER STATUS

5.1 INTRODUCTION

Population differences in taste perception have been the subject of study for many years. A discovery was made in the 1930's that there is a genetic difference in the way the population responds to thiourea containing compounds such as phenyl-thio-carbamide (PTC) and 6-n-propylthiouracil (PROP) (Blakeslee 1932). Variability in PROP taster status is, in part, explained by the TAS2R38 gene (Duffy, Davidson et al. 2004). There are 2 forms of this gene as a result of a single nucleotide polymorphism. The proline-alanine-valine (PAV) amino acid substitution is associated with 'tasting', whereas alanine-valine-isoleucine (AVI) associates with 'non-tasting'. This results in 3 common genotypes across the population. PAV homozygotes (PAV/PAV) are called 'super-tasters, heterozygotes (PAV/AVI) called 'medium-tasters' and AVI homozygotes (AVI/AVI) called 'non-tasters'. Duffy and Davidson (2004) found that PAV homozygotes taste greater bitterness from PROP than heterozygotes but it seems that other factors may be linked.

Fungiform papillae are most dense at the anterior of the tongue and house taste buds and receptor cells which respond to taste stimuli. A connection has been made that an increased number of fungiform papillae could lead to an increased sensitivity to all tastes (Miller and Reedy 1990; Zuniga, Davis et al. 1993) and somatosensation (Essick, Chopra et al. 2003; Hayes and Duffy 2007). Fungiform papillae density has also been found to be significantly higher in PROP super-tasters and women (Blakeslee 1932; Glanvill and Kaplan 1965; Bartoshuk, Duffy et al. 1994; Duffy and Bartoshuk 2000; Duffy, Davidson et al. 2004; Lanier,

Hayes et al. 2005; Driscoll, Perez et al. 2006; Hayes, Bartoshuk et al. 2008) suggesting that this could be the source of increased sensitivity to PROP/PTC as well as overall oral sensitivity (Duffy 2007; Duffy 2007; Bajec and Pickering 2008).

Studies have linked PROP taster status to increased sensitivity of the following;

- bitter (Hall, Bartoshuk et al. 1975; Bartoshuk 1979; Gent and Bartoshuk 1983; Bartoshuk, Rifkin et al. 1988; Mela 1990; Drewnowski, Henderson et al. 1997; Neely and Borg 1999; Chang, Chung et al. 2006; Dinehart, Hayes et al. 2006; Intelmann, Batram et al. 2009),
- sweet (Bartoshuk 1979; Gent and Bartoshuk 1983; Miller and Reedy 1990; Drewnowski, Henderson et al. 1997; Lucchina, Curtis et al. 1998; Chang, Chung et al. 2006),
- salty (Miller and Reedy 1990; Bartoshuk, Duffy et al. 1997),
- fat (Duffy, Bartoshuk et al. 1996; Eldeghaidy, Marciani et al. 2010),
- oral temperature (Manrique and Zald 2006; Bajec and Pickering 2008),
- oral irritants (Karrer and Bartoshuk 1991; Prutkin, Fast et al. 1999; Prescott and Swain-Campbell 2000; Duffy, Davidson et al. 2004; Duffy, Peterson et al. 2004)

This increased sensitivity has also been linked to hedonic responses and food preference in some studies (Glanvill and Kaplan 1965; Guinard, ZoumasMorse et al. 1996; Intranuovo and Powers 1997; Duffy and Bartoshuk 2000; Keller, Steinmann et al. 2000; Duffy, Davidson et al. 2004; Duffy, Peterson et al. 2004; Lanier, Hayes et

al. 2005). However, other studies have failed to find such correlations (Hall, Bartoshuk et al. 1975; Gent and Bartoshuk 1983; Leach and Noble 1986; Schifferstein and Frijters 1991; Drewnowski, Henderson et al. 1997; Drewnowski, Henderson et al. 1998; Delwiche, Buletic et al. 2001; Lim, Lenka et al. 2008). Conflicting results in the literature could be due to considerable variability in the methods used for classifying subjects into taster status groups between studies.

Green and Dalton et al (1996) introduced the general labelled magnitude scale (gLMS) as a more accurate measure of taste and smell between individuals and it has been widely adopted with positive results (Bartoshuk, Duffy et al. 2002). However, the concentration and procedure for administering PROP to subjects, along with the classification system of assigning subjects into 'taster groups' is still a source of variation. Studies vary in the procedure of PROP application from filter papers impregnated with supersaturated PROP to aqueous solutions of different concentrations used to determine detection thresholds or a single concentration applied either using a cotton swab to a particular part of the tongue or by whole mouth rinses. A unified approach towards research in this domain could improve disparity but there are still major challenges to overcome.

Research in this area is largely driven by the potential that food choice and preference could be influenced by individual or group differences (Dinehart, Hayes et al. 2006). The relationship between increased taste sensitivity and food liking or preference is complex involving many environmental, psychological and physiological factors. Trying to account for these factors presents a considerable challenge and it is likely that these could account for some of the

inconsistencies relating the PROP genotype to hedonic responses. Research focussing on beer is reviewed below.

5.1.1 PROP taster status and beer

Bitterness is a primary flavour component of beer and contributes to liking and consumption (Intranuovo and Powers 1997). The main compounds which impart bitterness in beer are the iso-α-acids, comprising of isohumulone, isoadhumulone and isocohumulone. The bitter receptors which respond to these and other bitter compounds found in beer have been identified from the group of ~25 which have been discovered to date. Encoded by the TAS2R gene family, hTAS2R1, hTAS2R14 and hTAS2R40 are activated in various combinations by the different chemical compounds eliciting bitterness in beer (Intelmann, Batram et al. 2009).

Research to date suggests that some bitter receptors recognise a wide variety of bitter compounds while other receptors are more specific (Maehashi and Huang 2009). It is therefore understandable why correlations between PROP taster status and general bitterness sensitivity are inconclusive. An investigation into the chemoreception and perception of isohumulones suggested that PROP has a different receptor mechanism to all other bitter stimuli, including isohumulones (Guinard, Hong et al. 1994). However, when the bitter intensity of a commercial beer (Pilsner Urquell) was rated in another study, super-tasters rated it significantly higher than non-tasters (Intranuovo and Powers 1997). In addition, the same study found that non-tasters self reported consuming significantly more beer when they first started drinking which agrees with other studies relating high levels of beer consumption

to non-tasting of PROP (Guinard, ZoumasMorse et al. 1996; Duffy, Peterson et al. 2004).

There are many factors which drive liking and consumption of beer and it is currently unknown whether these correlate with PROP taster status. The added complication of the physical effects of alcohol intake could override some of the negative associations with bitter taste so that consumption levels and liking do not correlate to taste sensitivity.

It is currently unknown if there is a relationship between the trigeminal system and PROP taster status. The trigeminal nerves receive information on sensation from the face. Free nerve endings from the lingual nerve branch surround the taste buds in the mouth (Whitehead, Beeman et al. 1985; Whitehead, Ganchrow et al. 1999). Increased activation of these nerves has been linked to a greater perception of oral burn from trigeminal stimuli such as alcohol and carbonation in PROP taster groups. Prescott and Swain-Campbell found that irritation from whole mouth rinses of 47.5% ethanol was more intense in tasters than non-tasters $P=0.026$ (Prescott and Swain-Campbell 2000). Duffy and Peterson found a link between activation from the chorda tympani (taste nerve) and greater intensity of an alcohol probe as well as higher fungiform papillae numbers and PROP intensities (Duffy, Peterson et al. 2004). Activation of the nerves surrounding, and in some people possibly innervating, the taste receptors has led to the further investigations of another population variation in oral sensitivity called thermal taster status.

5.1.2 Thermal taster status

In 2000, Cruz and Green published evidence of another taste phenotype that could be a better predictor of oral sensitivity than PROP. Thermal stimulation to the tongue was found to elicit a phantom taste in some individuals, classified as 'thermal tasters'. Sweetness was the quality most often tasted in these individuals when the tongue was warmed from 20-35°C, whereas reducing tongue temperature to ~20°C induced a sour taste that turned into saltiness at temperatures below 10°C (Cruz and Green 2000). The authors hypothesised that the mechanism could be a temperature sensitive chemosensory pathway. This was supported by discovery that the TRPM5 cation channel which responds to sweet, bitter and umami tastes is heat activated and highly temperature sensitive (Talavera et al 2005). Therefore the TRPM5 could mediate the phenomenon of thermal taste by depolarising the taste cells though thermal activation.

The discovery that thermal stimuli can act on the taste receptors has led to investigations comparing thermal tasters (TTs) and thermal non-tasters (TnTs). A series of experiments by Green and George (2004) revealed that thermal tasters were more sensitive to sucrose, saccharin, NaCl, citric acid, quinine sulphate, PROP, MSG, warming temperature, vanillin presented both orthonasally and retronasally and a sucrose-vanillin mixture. Variation in scale use between the groups may have been the causal factor; however the authors could not attribute this when a further experiment showed there was no significant difference in temperature ratings at non-gustatory sites (lip and hand). The results suggest that in addition to peripheral factors suggested previously, central neural processes may also contribute to individual differences in perception of both taste and flavour. Further research has

confirmed the increased taste sensitivity of TTs (Green 2005) but failed to find a link to other oral chemosensory sensations such as capsaicin and menthol (Green 2005). However, another paper found that TTs rated trigeminal (alum), gustatory (sucrose, NaCl) and olfactory (iron sulfate) stimuli significantly higher than TnTs (Bajec and Pickering 2008). Subsequent research by the same group has focussed on connections with food liking (Bajec and Pickering 2010) and alcoholic beverages such as wine (Pickering, Moyes et al. 2010) and beer (Pickering, Bartolini et al. 2010).

Bajac and Pickering (2010) investigated the difference between both thermal and PROP taster status for food liking, food neophobia, body mass index (BMI) and waist circumference. No significant differences were found between thermal taster groups for BMI, waist circumference and food neophobia. However differences were found between groups for food liking. TTs gave lower liking scores of cooked fruits and vegetables and the 'Mushy food group' (comprising of soft cheeses, hot cereal (oat and wheat), creamed corn, raw and cooked mushroom, cooked tofu, cooked peas, cooked squash, cooked turnip, cooked zucchini, cooked apples, raw avocado and raw banana) suggesting that they liked soft foods less than TnTs. This may be indicative of an increased sensitivity to oral tactile sensitivity which agrees with previous work by the same group using alum as an astringent tactile stimulus (Bajec and Pickering 2008). TnTs reported higher liking for a 'bitter correlation group' of foods (comprising of espresso, cooked turnip, cooked rutabaga, cooked mustard greens, cooked collard greens created by grouping bitter foods that were correlated with TTS). Conversely, two other studies by the same research group found no significant difference between TTs and TnTs for the overall liking of wine (Pickering, Moyes et al. 2010) and beer (Pickering, Bartolini et al.

2010) both of which have tactile elements (astringency and carbonation). However, in agreement with other studies, there was a trend that TTs rated the intensity of wine and beer attributes encompassing taste, flavour and tactile sensations higher than TnTs (Pickering, Bartolini et al. 2010; Pickering, Moyes et al. 2010).

The ability to taste PROP and/or the ability of a thermal stimulus to produce a taste response seems to function via independent mechanisms of separate genetic control as an interaction between PTS and TTS has so far not been found (Bajec and Pickering 2008). Current evidence would point to a correlation between PTS and increased fungiform papillae as the mechanism of increased taste response from tasters of PROP and this correlation has not currently been found with TTs (Bajec and Pickering 2008). The existing hypothesis for the mechanism of the TT advantage is that of a central gain mechanism (Green and George 2004; Green, Alvarez-Reeves et al. 2005; Bajec and Pickering 2008), the idea that 'something centrally' is accounting for the increased sensitivity in this group, as well as by variations in peripheral sensory factors such as co-innervations between taste and temperature receptors. Current data shows that thermal taste is associated with stronger perceptions of taste and flavour irrespective of which sensory nerve is stimulated (Green and George 2004).

The objectives of this study were to; (1) investigate the taste and thermal sensitivity of PROP taster status groups and thermal taster status groups, (2) screen subjects for further investigation into cortical response to taste and trigeminal stimuli.

5.2 MATERIALS AND METHODS

5.2.1 Subjects

52 subjects (32 female, 20 male) were recruited from a poster advertisement displayed at the University of Nottingham and the Queens Medical Centre, Nottingham. Approval from the University of Nottingham Medical School ethics committee was granted and written consent was obtained from all subjects. All 52 subjects completed 2 screening sessions; (1) to determine their sensitivity to tastants and PROP (1 h) and (2) to determine their thermal taster status (0.5 h). A disturbance allowance was paid for those who took part.

5.2.2 Scale use and training

The general Labelled Magnitude Scale (gLMS) was used to collect all psychophysical data (Green, Dalton et al. 1996; Bartoshuk, Duffy et al. 2002). The scale, shown in **figure 5.1**, consists of a vertical line with unequal quasi-logarithmic spacing between descriptors. Descriptors, 'no sensation', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable sensation of any kind' were placed at 0, 1.4, 6, 17, 35, 53 and 100% as determined by Green et al (1996). The numerical markers as shown in **figure 5.1** were not present on the scale.

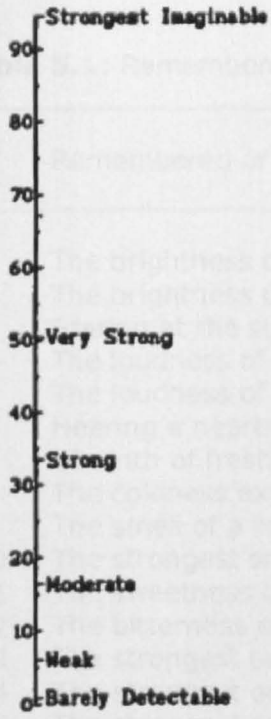

Figure 5.1: gLMS scale. Source: (Green, Dalton et al. 1996)

Training was given to increase understanding and validity of scale use (Bartoshuk, Duffy et al. 2002). All subjects were given a reference sheet with a gLMS presented in exactly the same way as subsequent test sheets. Subjects received verbal and written instructions that the top of the scale corresponded to the strongest imaginable sensation of any kind and were asked to write down what this was at the top of their reference sheet. Subjects were asked to rate a list of 15 remembered or imagined sensations (**table 5.1**) relative to their strongest imaginable sensation of any kind.

Table 5.1: Remembered or imagined sensations used in scale training

	Remembered or imagined sensation
1	The brightness of a dimly lit restaurant
2	The brightness of a well lit room
3	Staring at the sun
4	The loudness of a whisper
5	The loudness of a conversation
6	Hearing a nearby jet-plane take off
7	Warmth of freshly baked bread in your mouth
8	The coldness experienced sucking on an ice-cube
9	The smell of a rose
10	The strongest smell ever experienced
11	The sweetness of candyfloss
12	The bitterness of grapefruit
13	The strongest taste ever experienced
14	The strongest oral burn experienced
15	The strongest oral pain ever experienced

5.2.3 PROP taster status and tastant sensitivity

5.2.3.1 Stimuli

Taste stimuli were 0.32M sucrose (Tate and Lyle, UK) 0.56M NaCl (Sainsbury, UK), 56mM citric acid (Sigma Aldrich, UK), 1mM QHCl (Sigma Aldrich, UK) and 0.32mM PROP (Sigma Aldrich, UK). All solutions were prepared using deionised water the day before testing commenced. All stimuli were stored at 4-6°C prior to use and were served at room temperature.

5.2.3.2 Procedure

Each subject was instructed to rinse their mouth 3 times with deionised water. All stimuli were applied to the tongue by rolling a saturated cotton swab (Johnson and Johnson, New Jersey, US) across the tip of the tongue for approx 3s. The subjects were instructed to actively taste the stimulus between the tongue and

the hard palate using a gentle 'smacking' motion and rate the perceived intensity of the taste once it had reached its maximum using the gLMS provided. Separate gLMS were provided for each stimulus. Subjects were encouraged to refer to their reference sheet for guidance on where to rate the intensity of the taste. The four taste stimuli were presented first in a randomised order, PROP was presented last to avoid any cross over effects in PROP sensitive individuals. Subjects were given a 1 minute inter-stimulus-interval (ISI) and instructed to take longer if needed. During the ISI, subjects cleansed their palate with the deionised water and unsalted crackers (Rakusens, Leeds, UK) provided. After a 5 min break, the procedure was repeated to collect duplicate ratings of each stimulus. PROP taster status was defined based on the mean intensity ratings of PROP: pNTs < barely detectable (<1.4%) (n=19); pMTs above barely detectable (1.4%) but below moderate (17%) (n=18); pST > moderate (> 17%) (n=15) (Lim, Lenka et al. 2008).

5.2.4 Thermal taster screening

5.2.4.1 Procedure

A circular intra-oral ATS (advanced thermal stimulator) thermode (Medoc, Israel) with a truncated cone area of 28.26mm^2 at the top and 78.5mm^2 at the base (2mm height) (**figure 5.2a**) was connected to a PATHWAY pain and sensory evaluation system (Medoc, Israel) and controlled using PATHWAY software (version 4, Medoc, Israel) as shown in **figure 5.2b**.

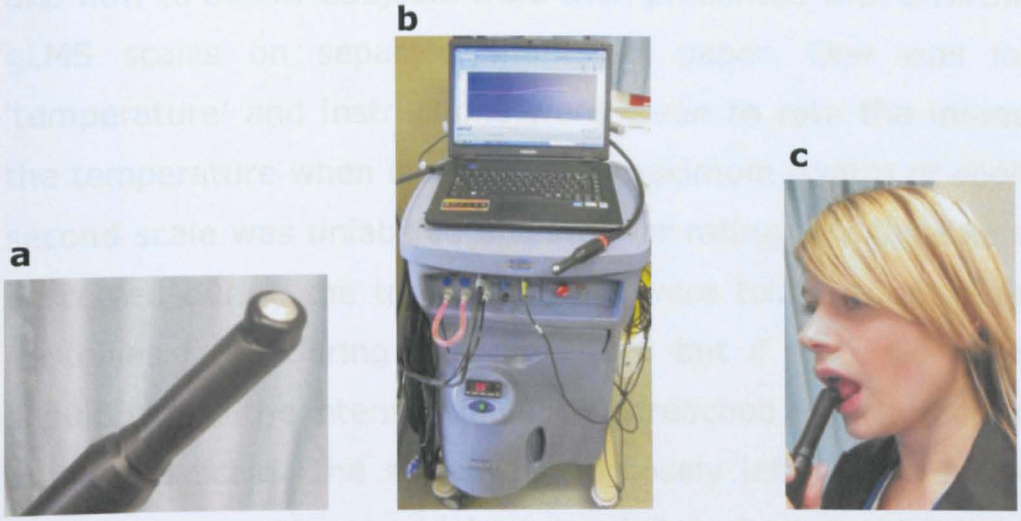

Figure 5.2: The Peltier thermode device. **(a)** the circular intra-oral thermode, **(b)** the Pathway pain and sensory evaluation system, **(c)** the inta-oral thermode in use.

The pain and sensory evaluation system consists of thermistors and Peltier elements. The thermistors sense changes in temperature and send feedback to the control unit which commands the heating or cooling of the thermode. The Peltier elements produce the temperature gradient between the upper and lower stimulator surfaces. The upper surface emanates heat produced by the external foil and the lower surface is cooled by the coolant which circulates to the external cooling unit.

The thermode was rinsed with 99% food grade ethanol (VWR International, Lutterworth, UK) between subjects and covered in a fresh piece of tasteless and odour free plastic wrap (TJMorris, Liverpool, UK) for each subject. The subjects were presented with their own reference sheet with the gLMS scale and their remembered or imagined sensations that they rated during training. A verbal reminder was given of how to use the scale and time was taken to re-familiarise them with the sensations rated in the training session and to ask any further questions about the scale

and how to use it. Subjects were then presented with a further two gLMS scales on separate sheets of paper. One was labelled 'temperature' and instructions were given to rate the intensity of the temperature when it reached its maximum (warm or cool). The second scale was unlabeled and was for rating any taste sensation perceived during the trials. Subjects were told that not everyone perceives taste during this procedure but if they did then they should record the intensity once it has reached its maximum on the unlabelled scale. The scale was purposely left unlabelled so that any taste sensation could be recorded and to avoid false report bias of thermal taste. Those who did sense a taste were asked to also record the taste quality (sweet, salty, bitter, sour, metallic etc).

The subject extended their tongue and the thermode was placed gently on the anterior tongue tip by the researcher. The subject was then asked to hold the thermode firmly in place as shown in **figure 5.2c**. The subject was asked to remove the thermode if the sensation became uncomfortable. The temperature ramp for all trials was 1°C/s. There was a minimum of 2 minutes between each trial to allow the tongue temperature to return to normal which was aided by the drinking of room temperature deionised water. Subjects were told to wait until the tongue temperature and sensation had returned to normal before proceeding onto the next trial. All trials were based on the method given by Bajac and Pickering (2008) except for one difference. Warming and cooling trials were carried out at one tongue location only, the anterior tip. This is because the tongue tip has been found to be most responsive to thermal taste (Cruz and Green 2000) and is where the taste papillae are most densely innervated (Shahbake, Hutchinson et al. 2005). A baseline trial was performed first to allow the subject to practice reporting the perceived temperature

and any other taste sensations. During this trial the thermode was applied to the tongue tip at body temperature (37ºC) and held for 10 s. Warming trials started at 35ºC, cooled to 15ºC and re-warmed to 40ºC and held for 1 s as shown in **figure 5.3a**. The cooling trial started at 35ºC and was cooled to 5ºC and held for 10s as illustrated in **figure 5.3b**.

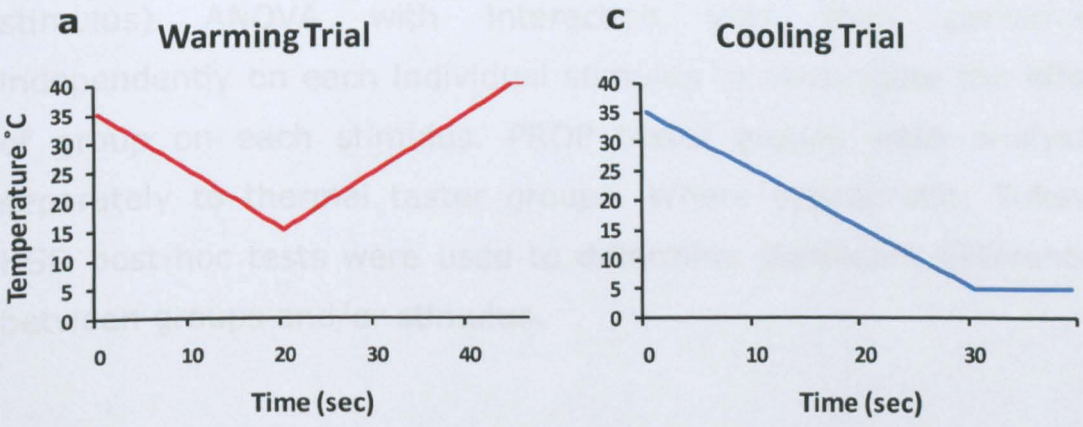

Figure 5.3: Graphic representations of the warming trial and cooling trials. **(a)** the warming trial started at 35°C, cooled to 15°C, re-warmed to 40°C and held for 1 s, **(b)** the cooling trial started at 35ºC and was cooled to 5ºC and held for 10 s.

Subjects were instructed to 'attend now' as soon as the warming began in the warming trial and the cooling began in the cooling trial and were asked to rate the temperature once it had reached its maximum perceived intensity (usually at the end of the trial). Trials were duplicated to provide replicate data but the subjects were unaware of this. Warming trials always preceded cooling trails to avoid possible adaptation from the intense, sustained cold stimulation (Green and George 2004). TTs were defined as those who reported a taste sensation during either the warming or cooling trials. Those who did not perceive any taste sensations were classified as TnTs.

5.2.5 Data analysis

All data was $\log_{10}$ transformed, with 0 ratings converted to 0.4 prior to transformation. A 3 factor ANOVA (group, replicate and stimulus) with interactions was performed on the log intensity ratings of the 4 taste stimuli, PROP and temperature to investigate overall effects of group, replicate and stimulus. A 2-factor (group, stimulus) ANOVA with interaction was then performed independently on each individual stimulus to investigate the effect of group on each stimulus. PROP taster groups were analysed separately to thermal taster groups. Where appropriate, Tukey's HSD post-hoc tests were used to determine significant differences between groups and/or stimulus.

5.3 RESULTS

5.3.1 PROP taster status

Fifty two subjects took part in the screening sessions of which 19 were PROP non-tasters (pNTs), 18 were medium-tasters (pMTs) and 15 were super-tasters (pSTs) as shown in **table 5.2**.

Table 5.2: A summary of subject's gender separated into PROP taster status and thermal taster status.

Subject Summary		Male	Female
TOTAL (n=52)		38%	62%
PROP taster status (n=52)	pNTs (n=19)	47%	53%
	pMTs (n=18)	44%	56%
	pSTs (n=15)	20%	80%
Thermal taster status (n=52)	TTs (n=12)	33%	67%
	TnTs (n=40)	40%	60%

A 3 factor ANOVA (group, replicate and stimulus) with interactions was performed on the log intensity ratings of the 4 taste stimuli, PROP and temperature. Results in **table 5.3** show that there were main effects of group, replicate and stimulus ($p<0.0001$).

Table 5.3: Mean logged intensity of group, rep and stimulus with significance level and post hoc groupings

Factor		Grand Mean	P Value
Group	pNT	1.027[a]	<0.0001
	pMT	1.235[b]	
	pST	1.396[c]	
Replicate	1	1.213[a]	<0.0001
	2	1.328[b]	
Stimulus	PROP	0.784[a]	<0.0001
	Sucrose	1.11[b]	
	NaCl	1.32[c]	
	Warming	1.33[c]	
	Quinine	1.34[c]	
	Citric Acid	1.4[c]	
	Cooling	1.57[d]	

There was also a group*stimulus interaction ($p<0.0001$) indicating that not all stimuli followed the same trend for each taster group. Further analysis revealed that the PROP ratings were the source of this interaction with much larger differences in ratings between groups than all other stimuli as illustrated by **figure 5.4**. This interaction is not surprising as the subjects were classified into taster status groups based on their response to the intensity of the PROP compound.

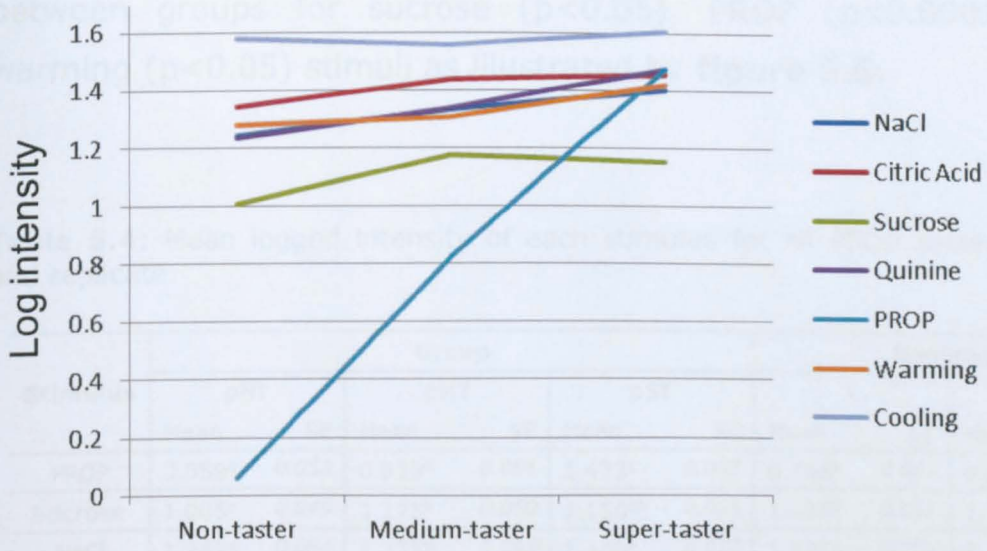

Figure 5.4: Mean scores of all stimuli rated by each PROP taster group illustrating the source of the significant group*stimulus interaction.

The significant group effect was driven by higher ratings of all stimuli by supertasters, followed by tasters, with non-tasters giving the lowest ratings which supports previous findings of an link between PROP taster status and oral sensitivity. The significant replicate effect was driven by higher ratings by made in replicate 2 compared to replicate 1 indicating a carry-over or learned effect. The significant effect of stimulus was driven by significantly different intensity ratings of PROP, sucrose and cooling stimuli as shown in **table 5.3**. Ratings for NaCl, quinine, citric acid and warming stimuli did not differ significantly.

To further understand the variance in the data, the data for each stimuli were analysed separately using a 2-factor (group and replicate) ANOVA and Tukey's honestly significant difference (HSD) post-hoc tests. Results, shown in **table 5.4** reveal that replicate 2 of all stimuli were rated higher than replicate one with sucrose ($p<0.05$), NaCl ($p<0.0001$) and citric acid ($p<0.05$) reaching significance. There were significantly different intensity ratings

between groups for sucrose ($p<0.05$), PROP ($p<0.0001$) and warming ($p<0.05$) stimuli as illustrated by **figure 5.5.**

Table 5.4: Mean logged intensity of each stimulus for all PROP taster groups and replicate.

Stimulus	Group						Replicate			
	pNT		pMT		pST		1		2	
	Mean	SE	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
PROP	0.059^{a}	*0.052*	0.819^{b}	*0.061*	1.473^{c}	*0.067*	0.748^{a}	*0.051*	0.819^{a}	*0.051*
Sucrose	1.005^{a}	*0.049*	1.175^{b}	*0.050*	1.150^{ab}	*0.055*	1.025^{a}	*0.042*	1.195^{b}	*0.042*
NaCl	1.242^{a}	*0.064*	1.335^{a}	*0.065*	1.400^{a}	*0.072*	1.235^{a}	*0.055*	1.416^{b}	*0.055*
Warming	1.281^{a}	*0.036*	1.307^{ab}	*0.037*	1.419^{b}	*0.040*	1.334^{a}	*0.031*	1.338^{a}	*0.031*
Quinine	1.232^{a}	*0.071*	1.340^{a}	*0.073*	1.475^{a}	*0.079*	1.276^{a}	*0.061*	1.422^{a}	*0.061*
Citric Acid	1.344^{a}	*0.043*	1.436^{a}	*0.044*	1.457^{a}	*0.048*	1.301^{a}	*0.037*	1.524^{b}	*0.037*
Cooling	1.576^{a}	*0.025*	1.555^{a}	*0.026*	1.606^{a}	*0.028*	1.576^{a}	*0.022*	1.581^{a}	*0.022*

Each stimulus was analysed separately. A different superscript letter denotes a significant difference within a row. pNT= PROP non-taster; pMT = PROP medium- taster; pST = PROP super-taster. SE = standard error

pNT's rated sucrose significantly lower ($p<0.05$) than pMT's but no significant difference was found between pNT's and pST's. As expected there was a significant difference between pNT's pMT's and pST's ($p<0.0001$) for PROP intensity scores. pNT's rated PROP lowest, followed by pMT's and pST's ($p<0.0001$). The warming temperature stimulus was rated significantly higher by pST's than pNT's ($p<0.05$), no significant difference was found between ratings made by pMT's and the other two groups for this stimulus. Ratings of quinine by pNT's were lower than pST's but did not quite reach significance ($p=0.062$).

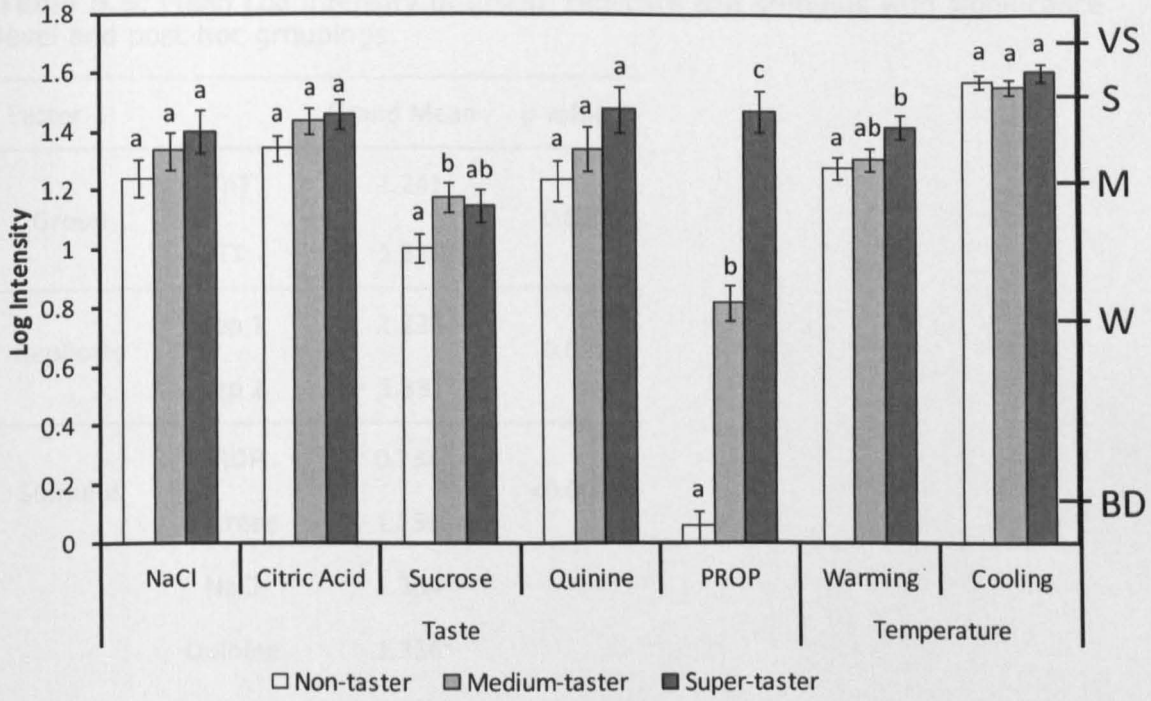

Figure 5.5: The effect of PROP taster status on log intensity of taste and temperature stimuli. The bars represent mean (logged) intensity ratings ± Standard error. BD= barely detectable, W= weak, M= moderate, S= strong, VS= very strong. Lower case letters denote significant differences between PROP taster groups within a stimulus.

5.3.2 Thermal taster status

Thermal screening revealed that 12 subjects were thermal tasters (TTs) and the remaining 40 subjects were thermal non-tasters (TnTs). Of these 12 thermal tasters, 4 were pNTs, 6 were pMTs and 2 were pSTs. A 3 factor ANOVA (group, replicate and stimulus) with interactions was performed on the log intensity ratings of the 4 taste stimuli, PROP and temperature. Results in **table 5.5** showed that there were main effects of group ($p<0.05$), replicate ($p<0.05$) and stimulus ($p<0.0001$).

Table 5.5: Mean Log intensity of group, replicate and stimulus with significance level and post-hoc groupings.

Factor		Grand Mean	p value
Group	TnT	1.241	0.024
	TT	1.318	
Replicate	Rep 1	1.225	0.002
	Rep 2	1.333	
Stimulus	PROP	0.734[a]	<0.0001
	Sucrose	1.136[b]	
	NaCl	1.354[c]	
	Quinine	1.356[c]	
	Citric Acid	1.441[c]	
	Warming	1.36[c]	
	Cooling	1.573[d]	

Subscript letters denote a different post hoc grouping

There were no significant interactions indicating that the trends seen in the data followed the same pattern. The significant group effect was driven by higher ratings by TTs compared to TnTs. The significant replicate effect was due to higher ratings made in replicate 2 compared to replicate 1, the same of which was observed in the PROP taster status data. Post-hoc tests revealed that the stimulus effect was due to significantly different ratings of PROP, sucrose and cooling stimuli compared to NaCl, warming, quinine and citric acid stimuli which did not significantly differ.

To further understand the variance in the data, the stimuli were analysed separately using a 2 factor (group and replicate) ANOVA. Results are shown in **table 5.6** and revealed that replicate 2 of

both citric acid and sucrose were rated significantly higher than replicate one ($p<0.05$).

Table 5.6: Mean (logged) intensity ratings of each stimulus for both groups and replicates

Stimulus	Group				Replicate			
	TT		TnT		Rep 1		Rep 2	
	Mean	*SE*	Mean	SE	Mean	SE	Mean	SE
NaCl	1.42^a	*0.08*	1.29^a	*0.04*	1.28^a	*0.06*	1.43^a	*0.06*
Citric Acid	1.50^a	*0.05*	1.38^b	*0.03*	1.34^a	*0.04*	1.54^b	*0.04*
Sucrose	1.19^a	*0.06*	1.08^a	*0.03*	1.06^a	*0.05*	1.21^b	*0.05*
Quinine	1.39^a	*0.09*	1.33^a	*0.05*	1.28^a	*0.07*	1.44^a	*0.07*
PROP	0.74^a	*0.14*	0.73^a	*0.08*	0.68^a	*0.11*	0.79^a	*0.11*
Warming	1.42^a	*0.05*	1.30^b	*0.03*	1.36^a	*0.04*	1.36^a	*0.04*
Cooling	1.57^a	*0.03*	1.58^a	*0.02*	1.57^a	*0.03*	1.57^a	*0.03*

Each stimulus was analysed separately. A different subscript letter denotes a significant difference within a row (stimulus) and column (group or replicate) . TT= thermal tasters, TnT = Thermal non-tasters. Rep1=replicate 1, Rep 2 = replicate 2. SE = standard error

TTs rated citric acid ($p<0.05$) and warming ($p<0.05$) significantly higher than TnTs and is shown by bold font in **table 5.6**. NaCl, sucrose and quinine show a trend of higher ratings by TTs but this failed to reach the significance level as shown in **figure 5.6**. Mean ratings of PROP and cooling did not follow this trend with just a 0.01 log score rating (approximately 1.024 in original scale) between the mean values of each group.

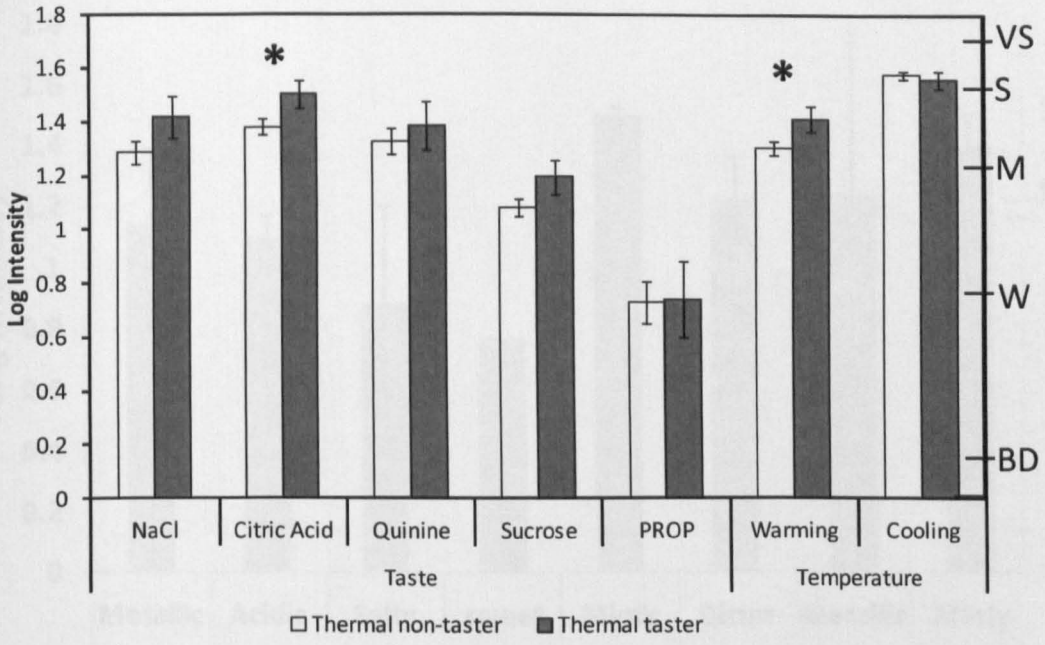

Figure 5.6: The effect of thermal taster status on log intensity of taste and temperature stimuli. The bars represent mean (logged) intensity ratings ± Standard error. * denotes a significant difference between groups for that stimulus ($P<0.05$)

Tastes experiences by TT subjects during oral temperature stimulation varied in quality and intensity. All mean taste intensities were rated between weak and moderate with an overall mean taste intensity rating of (log) 1.157 denoted by * on the secondary axis of **figure 5.7**. The most common taste experienced during the cooling trial was 'acidic' and this was sometimes reported along with 'metallic' (i.e. acidic/metallic) (**figure 5.7**). When subjects were probed to identify just one taste, they most often selected 'acidic'. In the warming trial each of the 3 tastes (bitter, metallic, minty) were reported an even number of times. Interestingly 'minty' was the strongest 'attribute' experienced by the subjects during both trials.

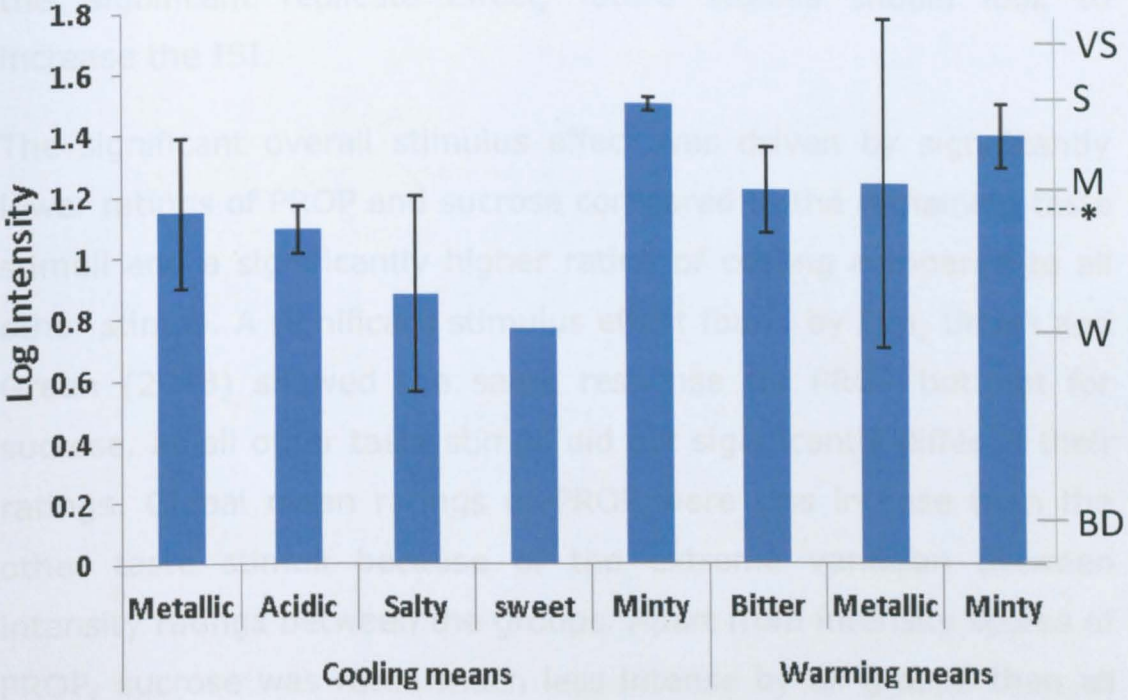

Figure 5.7: The taste quality and mean intensity experienced by thermal tasters during warming and cooling trials. Error bars show standard error. BD = barely detectable, W = weak, M = moderate, S = strong, VS = very strong and * = overall mean taste intensity.

5.4 DISCUSSION

5.4.1 PROP taster status and taste sensitivity

Significantly higher intensity ratings for replicate 2 than replicate 1 could be explained by a stimulus carry over effect or a learned effect. A significant replicate effect was also seen with results from Lim, Urban and Green (2008) using the same method to determine PROP taster status and taste sensitivity, although, when they analysed results independently for stimulus, there was no significant difference between replicates for the 5 taste stimuli (NaCl, Sucrose, Citric acid, QHCl and PROP). Whereas in the current study, replicate 2 was rated significantly higher than replicate 1 for sucrose, NaCl and citric acid. The inter-stimulus-interval (ISI) was the same in both studies. However, in light of

this significant replicate effect, future studies should look to increase the ISI.

The significant overall stimulus effect was driven by significantly lower ratings of PROP and sucrose compared to the remaining taste stimuli and a significantly higher rating of cooling compared to all other stimuli. A significant stimulus effect found by Lim, Urban and Green (2008) showed the same response for PROP but not for sucrose, as all other taste stimuli did not significantly differ in their ratings. Global mean ratings of PROP were less intense than the other taste stimuli because of the extreme variation between intensity ratings between the groups. Apart from intensity scores of PROP, sucrose was rated much less intense by all groups than all other stimuli as illustrated by **figure 5.5**. This was not the case with the study by Lim et al (2008) which is surprising considering the same concentrations were used. The lower intensity rating of sucrose could be due to hedonic bias, whereby subjects were influenced by the pleasantness of the stimulus. The training given on scale usage did not mention the importance of decoupling hedonic bias to the subjects and would be recommended for future studies.

Stimulus intensity ratings were analysed independently between groups and significant differences were found for PROP, sucrose and warming stimuli only. PROP intensity was positively correlated with taster status. Sucrose was rated significantly higher by pMT's compared to pNT's. Warming was rated significantly higher by pST's compared to pNT's. Lim et al (2008) combined intensity ratings from pMT's and pST's into a 'taster' group and compared their taste intensity results to pNT's using a student t-test. Quinine was the only stimulus to be rated significantly higher by the 'taster'

subjects. When the group was split into pMT's and pST's, the difference between intensity ratings, analysed by t-test, found that pST's rated all 4 taste stimuli (and PROP) significantly higher than pMT's indicating that the PROP genotype advantage is much greater for pST. In the current study, ANOVA did not reveal any significant differences between pMT's and pST's for any of the prototypical taste stimuli (sucrose, citric acid, NaCl or quinine) tested here. The t-tests carried out in the Lim, Urban and Green (2008) paper may have increased the probability of finding significant differences between groups for taste stimuli.

The warming stimulus was rated significantly higher by pST's than pNT's but no significant difference was found between groups for the cooling stimulus which is contradictory to other work (Manrique and Zald 2006), although much higher target temperatures were used for the cooling stimuli (21, 24 and 27°C) than in the current study (5°C). Response to both warming and cooling temperature stimuli was investigated between PROP taster groups at 3 different locations on the tongue by Bajac and Pickering (2008). Results showed that pST's rated all temperature stimuli significantly higher than both pNT's and pMT's. The increased temperature sensitivity in pST's was thought to be linked to fungiform papillae density which was higher in pST's compared to the other two groups (Bajec and Pickering 2008). Fungiform papillae density was not measured in the current study so this hypothesis cannot be investigated but it is likely since the nerve endings that respond to temperature and other trigeminal stimuli are innervated with the taste receptor cells (Whitehead, Beeman et al. 1985; Whitehead, Ganchrow et al. 1999). An increased number of fungiform papillae in pST's compared to pMT's should therefore result in an increased number of trigeminal receptors responding to temperature and contributing

to the increased intensity response. However, the link between PROP taster status and fungiform papillae density is not fully conclusive and the genetic control for fungiform papillae density is not clear (Duffy, Davidson et al. 2004).

The significant group effect in PROP ratings is due to the genetic ability to taste PROP as previously discussed. pNT's had the lowest ratings, followed by pMT's and then pST's which confirms the genetic effect. Sucrose and warming were also rated significantly differently between the groups. It is interesting to note that sucrose was the taste stimulus rated overall least intense and warming was the temperature stimulus rated overall least intense. Therefore, it may be possible that the PROP advantage is greater at lower stimulus intensities. This could be a plausible explanation considering the connection between PROP taster status and fungiform papillae density. For example, in percentage terms, a difference in fungiform papillae density could bring about a greater difference in receptor activation and therefore signals sent to the brain, resulting in greater differences in perception at lower stimulus intensities between groups. Higher stimulus intensities may saturate the receptors and subsequent signalling to the brain could bring about a perceptual response which is similar between groups. Another explanation could be that sucrose and warming stimuli are generally more pleasant stimuli than the others and the PROP advantage is more susceptible to this.

pST's rated all stimuli higher than pNT's however, the magnitude of the difference between the groups is stimulus dependant (**table 5.4**). As expected, the difference between group mean intensity ratings for PROP was much larger than any other stimulus but when comparing the taste stimuli alone there was a larger

difference between intensity ratings between the pNT's and pMT's. However, with the temperature stimuli, there was a greater magnitude of difference between pMT's and pST's. The mechanism for the PROP taster advantage is reported to be a combined effect of allelic variation of TAS2R38 and an increased number of fungiform papillae on the anterior tongue. Tasters must have at least one PAV allele but it is the density of fungiform papillae on the anterior tongue that separates pMT's and pST's (Bartoshuk, Duffy et al. 1994; Delwiche, Buletic et al. 2001; Duffy, Davidson et al. 2004). It is possible that the presence of the functional receptor for PROP contributes to increased taste sensitivity and would suggest why there was a greater difference between pNT's and pMT's for taste stimuli found here, whereas the increased density in fungiform papillae between pMT's and pST's (found in other works) accounts for the increased temperature sensitivity found here and possibly other stimuli sensed by the trigeminal nerve. This hypothesis is supported by temperature intensity ratings collected by Bajec and Pickering (2008) where the magnitude of difference was greater between pMTs and pSTs, but not the taste intensity ratings collected by Bajec and Pickering (2008) and Lim, Urban and Green (2008) where greater differences were also seen between pMT's and pST's.

The mechanism for an increased perceptual response by tasters of PROP (both pMTs and pSTs) to taste stimuli and temperature found in the current study could be a combination of anatomy and receptor genetics which would both increase signal response and central activation. Neurophysiological studies into brain activation between taster groups of various taste and tactile stimuli would increase understanding of the mechanisms that give the PROP taster advantage.

5.4.2 Thermal taster status and taste sensitivity

A significant effect that replicate 2 was rated higher than replicate 1 suggests the same carry over effect as discussed in the previous section. In addition, the significant global effect of stimulus showed that PROP, sucrose and cooling stimuli were rated significantly differently to all other stimuli and is the same result found in the PROP group analysis. The parity between the replicate and stimulus effect seen between groups is not surprising considering this group comprises of the same subjects but classified for thermal taster status as opposed to PROP taster status.

PROP taster status was spread relatively evenly across the thermal taster group and there was no significant difference found between PROP intensity ratings between thermal groups indicating that the two markers of oral sensitivity function independently. This agrees with some research (Bajec and Pickering 2008; Bajec and Pickering 2010) but disagrees with others (Green and George 2004). Green & George (2004) tested PROP sensitivity between thermal taster groups on both the front and back of the tongue. At both locations, PROP was rated significantly higher in intensity by TTs but the magnitude of difference between the groups was much larger on the back of the tongue than on the front. The same was true for another bitter stimulus, quinine, but not for sucrose, NaCl or citric acid taste stimuli. In the current study, PROP was applied to the anterior tongue and it is the posterior tongue that is densely populated with circumvallate papillae (Chandrashekar, Hoon et al. 2006) which express all 25 TAS2R (bitter receptor) genes (Behrens, Foerster et al. 2007). Therefore, future investigations should test for sensitivity to bitter tastants using either whole mouth rinses or

if using cotton swabs, both the anterior and posterior tongue should be tested.

A significant group effect revealed that TTs rated all stimuli significantly higher than TnTs which agrees with other studies (Green and George 2004; Bajec and Pickering 2008). When each stimulus was subjected to independent 2-factor (group and stimulus) ANOVA, the citric acid taste stimulus and the warming temperature stimulus were the only stimuli to reach significance, although all other taste stimuli followed the same trend. However, the mean group intensity ratings of the cooling stimuli did not differ between groups; in fact there was only a 0.01 log difference (1.024 in original scale) between mean group ratings (**table 5.6**).

Previous studies have found conflicting evidence regarding the impact of the TT advantage on trigeminal stimuli. Burning, stinging and prickling induced by capsaicin and menthol were not rated differently by thermal groups in a series of experiments by Green et al (2005) but the astringency of alum and temperature of warm and cold stimuli were rated significantly higher by TTs in a study by Bajec and Pickering (2008). The log mean intensity of warm stimuli was rated significantly higher by TTs at three locations on the tongue; the tip, and the left and right sides, which is in agreement with results here on the tongue tip. However, the cooling stimulus was only significant at the right and left sides and no significant difference was found at the tongue tip which again agrees with results found here.

Thermal tasters seem to perceive tactile and temperature stimuli more intensely which may influence food liking and preference. Thermal tasters have reduced liking for soft foods (Bajec and Pickering 2010) providing evidence that differences between TTs

and TnTs could be texturally driven. The mechanism for this would not appear to be associated with fungiform papillae density as no correlation has been found previously (Bajec and Pickering 2008). Differences in trigeminal sensitivity may differ between the two groups based on interaction with a taste stimulus. Carbonation and fullness were rated higher by thermal tasters in a study on beer (Pickering, Bartolini et al. 2010) and astringency was rated significantly higher in red wine (Pickering, Moyes et al. 2010) but when capsaicin and menthol were investigated in isolation, no significant differences were found between groups. A similar interaction mechanism has been proposed for increased aroma sensitivity in TTs (Green and George 2004).

The magnitude of the difference between intensity ratings between thermal taster groups is lower than those found in other studies (Green and George 2004; Bajec and Pickering 2008). In the current study the TT screening test involved thermal testing at just one site on the tongue (anterior tip), whereas other studies also tested to the right and left of the anterior tip therefore providing stricter criteria for TT classification. Sensitivity to thermal taste is not uniformly distributed as with chemical taste (Cruz and Green 2000) and the best sites for thermal sweetness (sweet-best sites) have been found on the tongue tip whereas sites for thermal sourness (sour-best sites) have been found nearly always lateral to the tip (Cruz and Green 2000). Cruz and Green (2000) found that when sucrose was applied to sweet-best sites in TTs, ratings were higher than when applied to sour-best sites. In addition, in the study by Green and George (2004) taste intensity to sucrose, sodium saccharin and NaCl was tested at the site most sensitive to thermal taste, with all TnTs being tested at the tongue tip which may have increased the difference between results. Therefore

classifying subjects into TT groups based upon thermal stimulation at just one tongue location and testing oral sensitivity at the same location may have led to wrongly classifying some subjects in the present study and thus reducing the magnitude of the difference between groups. It would be recommended to test 3 tongue locations in future studies and to reject subjects if a clear classification cannot be made. Nevertheless, this study demonstrates that time or cost restrictions make it possible to test at the tongue tip only for quick screening purposes.

Another explanation for the magnitude of difference between groups in different studies could be dependent upon stimuli concentration. Green and George (2004) tested the sensitivity to the same stimuli as in the current study using cotton swabs but at lower concentrations (concentrations taken from the text as opposed to the graph labels in Green and George (2004) as there is a discrepancy between the two). Greater differences were found between groups for taste sensitivity than in the present study indicating that as the concentration of the stimulus increases, the TT advantage decreases. Another study by Green et al (2005) using very similar concentrations of the same taste stimuli as in the present study and applied in the same way, showed similar results in terms of magnitude of difference between the groups.

Taste intensities experienced during temperature stimulation in thermal tasters (**figure 5.7**) were within the same intensity range (between weak and strong on the gLMS scale) as those experienced during the taste sensitivity testing (**figure 5.6**). This suggests that trigeminal (temperature) nerves may be capable of delivering a taste sensation that is equally comparable in intensity to those achieved by the taste compounds. The impact this has on

the way thermal tasters experience more complex foods and beverages is yet to be understood.

The quality of the taste varied between subjects and each of the tastes reported during the warming trial were given an equal number of times with no one taste dominating. Cruz and Green (2000) found that 'sweet' was the most common taste reported by thermal tasters during warming trail which was not the case in the present study. However, the term 'minty' was reported in both warming and cooling trials and could be a combination of tingly sensation from the swift temperature change combined with a sweet taste. The most common taste experienced during the cooling trial was 'acidic' which agrees with other trials, followed by 'metallic'. Metallic has not been a taste which has been reported by other authors. This may be due to the fact that in this study the subjects were free to place their own label on the taste sensation experienced so as to not introduce response bias. Whereas in other studies, subjects have been provided with gLMS scales labelled with the basic tastes (sweet, salty, sour, bitter, (umami) and 'other'). In this case, 'metallic' could in fact be the label put to acidic taste because it was sometimes accompanied by the term 'acidic' in the subject's initial description (i.e. acidic/metallic). When the subject was asked to use just one word to describe the taste, they most often chose 'acidic' for the cooling trial. In the cases where 'metallic' was chosen it is likely that the subjects used this term to describe a taste that they were having difficulty identifying because they could see the metal contact plate of the thermode, thus responding to the visual cue of the thermode. It is not possible for a metallic taste to have transferred from the thermode contact plate to the subject because it was covered with plastic film wrap.

Subjects could record the taste sensation at any point during the trails and the specific temperature at which the taste was perceived is unknown as this served as a screening test and so full analysis of the differences between taste quality and warming and cooling cannot be made.

5.5 CONCLUSIONS AND SUMMARY

An increased sensitivity of general bitterness, overall taste sensitivity or indeed overall oral sensation in different PROP groups has been extensively researched. This study confirms that PROP and thermal taster status are different markers of genetic taste variation in the population. It is possible that the disparity in results from studies investigating PROP taster status could occur from differences in stimuli concentrations and application method. Results to date suggest that PROP super-tasters sense oral stimuli more intensely due to a combination of a dominant PAV allele and high fungiform papillae density. The genetic mechanism for fungiform papillae density is currently not known but seems to be linked to the sensation of tactile stimuli. Further work should investigate PROP sensitivity and sensory interactions between modalities. Thermal taster status was a significant predictor of sensitivity to taste stimuli and further work should investigate gustatory-trigeminal interactions to investigate the mechanism of the thermal taste advantage. If the intensity of all oral sensations is greater in thermal tasters due to activation of both trigeminal and taste fibres which are co-innervated and intertwined then it is likely that activation in cortical areas involved in gustatory and somatosensory processing will be also increased. Psychophysical studies rely on the subject to verbalise these perceptions. However,

neuroimaging techniques can provide a detailed account of cortical activation which can be correlated to genetic phenotype, perception and preference if measured concurrently. An increased knowledge of neural and cortical responses between groups is needed to further understanding in this domain and is explored in the next chapter.

Chapter 6

6. THE CORTICAL RESPONSE OF CARBONATION ON TASTE AND VARIATION WITH TASTE PHENOTYPE

Carbonation was found to reduce sweetness perception in a model beer system (chapter 4) and it is unknown whether this is the result of a peripheral or cortical interaction. In addition, chapter 5 explored variation of taste phenotype and found differences between groups for taste and temperature perception. The objectives of this study were to use functional magnetic resonance imaging (fMRI) to; (1) investigate the cortical response to (sweet) taste and the effects of carbonation and (2) compare the cortical response between taste phenotype groups. The following sections introduce magnetic resonance imaging as a tool to investigate activation from sensory neurons.

6.1 INTRODUCTION TO MRI

Magnetic resonance imaging (MRI) uses strong magnetic fields, expressed in units of Tesla, which can be manipulated to create images of specific biological tissue. Atomic nuclei in the tissue absorb electromagnetic energy emitted by the scanner at a particular frequency. This absorbed electromagnetic energy is then released by the nuclei, the intensity of which is dependent upon the number and type of nuclei present in the tissue. To create the images, the scanner uses a complex sequence of magnetic gradients and radiofrequency (RF) pulses, together comprising what is known as a pulse sequence, from which an image is formed. The type of imaging pulse sequence used depends upon the different tissue properties to be detected. For example, a different pulse sequence would be used to create structural images of the foot or brain, compared to functional images of the brain. MRI can be used to create high resolution structural (anatomical) images of

the brain to define anatomy using pulse sequences which take a number of minutes to acquire an image. Functional studies (which are termed functional Magnetic Resonance Imaging (fMRI)) on the other hand, rapidly acquire a typically coarse image on the order of milliseconds to measure short term physiological changes associated with active functioning of the brain. fMRI can provide valuable information on where particular mental processes occur and the spatial patterns and intensities of activation associated with them. The following sections will describe the basic principles of magnetic resonance imaging including pulse sequences and image formation. This is followed by an outline of the use of Blood Oxygenation Level Dependent (BOLD) contrast in functional MRI and an overview of data collection and analysis methods. The introduction will conclude with a review of the cortical representation of taste and trigeminal stimuli.

6.1.1 Basic principles of Magnetic Resonance

Magnetic resonance imaging relies on the fact that atomic nuclei possess four fundamental physical properties: mass, electric charge, spin and magnetic moment. Nuclei which contain an odd number of protons, or an odd mass number (number of protons and neutrons) possess intrinsic spin, and have spin angular momentum (**J**). A hydrogen nucleus (^{1}H, proton) possesses spin 1/2 and this gives rise to a magnetic moment (μ) which is described by **eqn. 6.1**. γ is the gyromagnetic ratio, which is a fundamental property of the nucleus.

Eqn. 6.1 $\mu = \gamma \mathbf{J}$

Hydrogen nuclei give the greatest nuclear magnetic resonance (NMR) signal because of the large natural abundance in biological tissues (99.8%), and their protons have the largest gyromagnetic ratio (γ) which is 42.58 MHz/Tesla. When placed in a magnetic field, protons with magnetic moment μ precess clockwise around an axis parallel to the main magnetic field, $\mathbf{B_0}$ (**figure 6.1**). The angle between the proton's spin axis and that of the main magnetic field is determined by the proton's angular momentum (**J**).

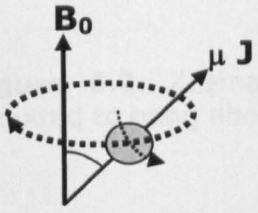

Figure 6.1: A schematic illustration of a spin (shaded sphere) which has a magnetic moment (μ) and angular momentum (**J**), the spins precessing about an axis parallel to the static magnetic field ($\mathbf{B_0}$) illustrated by the circular dashed arrow. The spin direction is illustrated by the small dashed arrow. The bold line shows the direction of the static magnetic field ($\mathbf{B_0}$) pointing along z.

The resonant frequency of a spin (ω) within a magnetic field is described by the Larmor frequency (**eqn. 6.2**). The Larmor frequency (ω_L) is determined by the gyromagnetic ratio multiplied by the static magnetic field strength, for protons at 3T (as used in this study) the precession frequency is approximately 128 MHz.

Eqn. 6.2 $\omega_L = \gamma B$

In a magnetic field, in the z-direction, **J** is quantized and can take values of $J_z = \hbar m_I$ where $\hbar$ is Planck's constant divided by 2π and m_I is the spin state. For hydrogen, a spin can take one of two spin states, either spin-up (m_I =+1/2) or spin-down (m_I =-1/2). Therefore a proton can take one of two energy states, with the

energy difference (ΔE) between the two spin states termed the Zeeman splitting and given by the Zeeman equation (**eqn.6.3**).

Eqn. 6.3 $$E = -\gamma m_I \hbar B$$

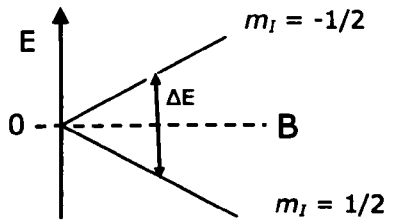

Figure 6.2: Zeeman splitting – a schematic representation of the energy required to bring about a change in spin state.

As static magnetic field (B_0) increases, this leads to an increase in the energy difference (ΔE) between the two energy states, as illustrated by **figure 6.2**. Both field strength and temperature affect the net magnetization.

So far, a single spin has been considered, but there are a large number of spins in a sample. In the absence of a magnetic field and at thermal equilibrium, these spins are uniformly distributed between the two energy states resulting in zero net magnetization. If the temperature is reduced to zero kelvin (K) and a magnetic field is applied, the net magnetisation ($M = \sum\mu$) is increased due to 100% alignment of spins with the static magnetic field (in 'spin-up' low energy state). Reducing the temperature is a method which can be used only in non-biological samples to increase the net magnetisation. However, in human MRI, it is not possible to reduce body temperature to zero kelvin, so magnetic field strengths are increased to bring about a small, but measurable increase in net magnetization by causing a difference in the

populations of spins in the two energy levels. With an excess of spins in the 'spin-up' state, this gives rise to a net magnetization parallel to the static magnetic field (B_0) called the bulk or net longitudinal magnetization (M_0). However, only a small proportional of the spins are in the 'spin-up' state and so the net magnetisation is very small. For protons at 3T and 300K, 10 parts per million (ppm) of the total spins are in the 'spin-up' state which is aligned with the static field. By applying RF pulses this magnetisation can be knocked into the xy-plane perpendicular to B_0, this is known as the transverse plane and the corresponding magnetisation the transverse magnetisation (M_{XY}).

6.1.1.1 Excitation: radio frequency pulse

When a subject is placed in a magnetic field, such as that created by an MR magnet, there is an excess of spins in the 'spin-up' state resulting in net magnetization (M_0) which is parallel to the static magnetic field (B_0). When the net magnetization of spins (M_0) is parallel to the static magnetic field (B_0), the net magnetisation cannot be measured because it is very small and swamped by the static field (which is of the order of Tesla). Therefore, a radiofrequency (RF) magnetic field of the appropriate electromagnetic energy is applied to cause transitions of spins between the Zeeman energy levels and knock the magnetisation from the longitudinal plane (M_Z) into the transverse plane (M_{XY}). The energy of this RF pulse must match the Zeeman energy splitting $\Delta E = \gamma\hbar B_0 = \hbar\omega$ by applying an RF pulse of frequency $\omega = \gamma B_0$. Thus these RF fields are not static, they rotate at the Larmor frequency (ω_L). The angle through which the magnetisation is flipped (flip angle, α) is determined by the amplitude of the RF

pulse (B_1) and the duration for which it is applied (τ), and is expressed by $\alpha = \gamma B_1 \tau$

This RF pulse is created by a special 'RF coil' which is tuned to the appropriate frequency and can be used to generate a field of amplitude (B_1) which is circularly polarised, rotating at the Larmor frequency in the xy-plane and is therefore known as the rotating reference frame with co-ordinates x', y' and z (the magnetic field of the scanner is known as the laboratory reference frame with the co-ordinates, x, y and z.) RF coils deliver electromagnetic waves (energy) which are oscillating at the Larmor frequency to the spins. This changes the net distribution of spins between the low energy ('spin-up') and high energy ('spin-down') states, resulting in more spins in the high energy state. If viewed in the rotating frame, spin precession and the oscillatory component of the excitation pulses can be ignored as they become stationary. The net magnetization can then be thought of as stationary along the z direction within the rotating frame. An excitation pulse (B_1) at the Larmor frequency would rotate the magnetization vector (M_0) from the z-direction (aligned with the static field) into the transverse (xy) plane.

This RF pulse has two effects: (i) it alters the population of spin states, and (ii) it brings spins into phase, resulting in the net magnetization precessing about the applied RF pulse B_1 in the x-y plane. The RF pulse required to generate equal numbers of nuclei in each energy state and flip magnetization into the xy-plane is known as a 90° excitation pulse, this also acts to bring all spins into phase. If the RF pulse were applied for a longer duration, to the point where the proportion of spins is exactly opposite to the original proportions (termed inverted), then this is called a 180°

inversion pulse. This is often used to increase contrast on some anatomical images.

When the electromagnetic energy is turned off, the excess spins in the higher energy level must return back to the lower level (back to equilibrium), and the spins must get back out of phase. When the high energy spins fall back to the low energy state, they emit energy which is equal to the energy difference of the two states (energy corresponding to the Larmor frequency). During this period, the reduction in transverse magnetization can be detected using an RF coil tuned to the Larmor frequency and because the frequencies of excitation and reception/detection are identical, the same RF coil can be used for both processes.

6.1.1.2 Relaxation of MR signal

The detected MR signal does not remain stable for long, resulting in two processes: (i) recovery of longitudinal magnetization (T_1, as illustrated by **figure 6.3**), and (ii) loss of coherence of transverse magnetisation (T_2/T_2^*, as illustrated by **figure 6.4**), these process are termed relaxation. Different tissues (for example, grey or white matter) recover from an RF excitation pulse differently and therefore give different intensities in signal. This is how MR images provide contrast. Depending on the tissue of interest, one of the relaxation parameters can be targeted to collect images which are sensitive to the required properties of the tissue.

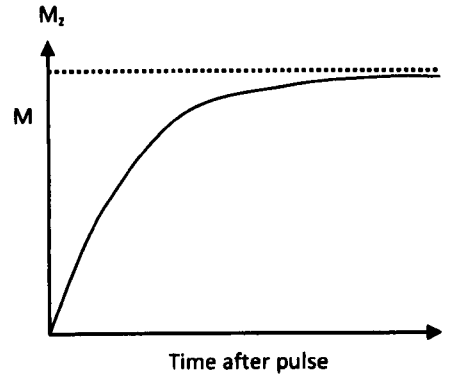

Figure 6.3: Longitudinal recovery described by the T_1 relaxation time $M_z(t) = M_0(1 - e^{-t/T_1})$

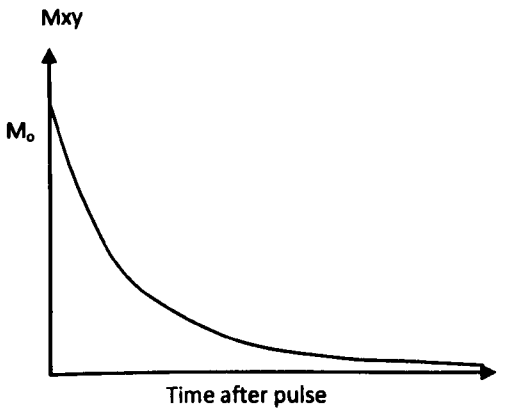

Figure 6.4: Transverse decay described by T_2 relaxation time $M_{xy} = M_0 e^{-t/T_2^*}$

Relaxation processes govern how much MR signal can be acquired following a single excitation pulse because MR data can only be collected during the transverse relaxation period/window, which is short (typically of the order of a milliseconds in tissues) and is governed by the relaxation time constant T_2 or T_2*. In addition, the T_1 component governs how many images can be collected, because for optimal signal, longitudinal relaxation must be fully recovered before another excitation pulse can be delivered (this is of the order of seconds for tissues). The time interval between excitation

pulses is known as the repetition time or TR. Therefore MR signal depends on the original bulk magnetization (M_0) but also on the properties of the tissue being imaged, and different tissues have different T_1 and T_2/T_2^* time constants. As field strength increases, T_1 lengthens and T_2/T_2^* shortens. The time interval between excitation and data acquisition is called the echo time or TE.

Longitudinal relaxation (governed by the T_1 relaxation time) is the process by which the spin system returns back to equilibrium after absorption of RF energy. This is caused by dipole-dipole interactions between spins and causes the field between protons and neighbouring molecules to be affected by each other generating a randomly fluctuating magnetic field at the Larmor frequency.

The time constant which describes the decay of transverse magnetization due to spin-spin interactions (accumulated phase differences) is the T_2 decay. T_2 decay varies in time and cannot be rephased. The time constant which describes local magnetic field in-homogeneities is called T_2' decay, which in contrast to T_2, is static in time and can be rephased. The time constant which describes the decay of transverse magnetisation due to the combined effects of spin-spin interactions and local magnetic field inhomogeneities is called T_2^* decay, and T_2^* is always faster than T_2 decay. Following a 90° pulse, all spins are brought into phase, $M_{xy}= M_o$. M_{xy} is now rotating at the Larmor frequency, however, this is dependent upon the local magnetic field which varies across the sample and causes dephasing of spins and reduced M_{xy}. After a given time, spins become completely dephased and $M_{xy}=0$. In tissues with short T_2^*, signal decays very quickly, leading to loss of signal.

MRI images are dependent upon creating contrast between a wide range of different tissues. Static contrasts are sensitive to the number of nuclei (protons) and the relaxation properties (T_1, T_2, T_2^*). As well as using natural contrast from utilising the difference in relaxation times from different tissues, contrast agents can be injected to provide additional contrast in tissues. However, these are not used for fMRI and therefore will not be discussed further.

Following an RF pulse, the free induction decay (FID) is the basic form of signal detected in MR, this signal oscillates at the Larmor frequency and decays exponentially with a decay time of T_2^*. This received signal is used to spatially encode images.

6.1.2 Image formation

Gradients are spatially varying magnetic fields which cause nuclei in different spatial locations to precess at different rates (frequencies). They are deliberately applied to vary the Larmor frequency across the sample, and so by applying three gradient fields in orthogonal directions these can be used in image acquisition to resolve information about the spatial position of nuclei in three dimensions.

Gradient magnetic fields applied along the x, y,and z directions (**figure 6.5**) change the strength of the magnetic field in each of the three directions (with the direction of the static magnetic field always along z). Images are created by utilising a sequence of gradient field changes and RF pulses (pulse sequence). Image formation using echo planar imaging (EPI) (as used in this study) first involves slice selection, to select a slice of the object being imaged, followed by frequency encoding and phase encoding to resolve the in-plane signals within the slice.

6.1.2.1 Slice selection

To select a slice, a magnetic field is applied in the slice direction (e.g. in z which would result in an axial or transverse slice) which causes the Larmor (spin precession) frequency to vary along z. **Figure 6.5** illustrates the x, y and z directions. If a gradient is positive, then spins towards the top of the brain would precess more rapidly (greater than ω_L) than spins towards the bottom (lower than ω_L), resulting in a varying magnetic field strength along that axis, as illustrated by **figure 6.5**.

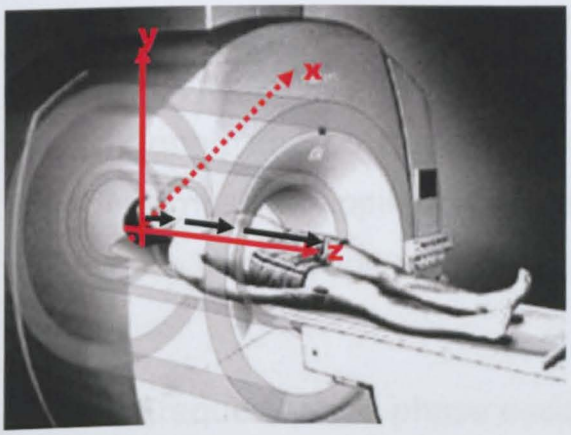

Figure 6.5: Schematic representation of the x, y, and z axes shown in red with an illustration of a gradient along the z axis shown in black. The size of the black arrows illustrates the strength of the magnetic field as a result of the applied gradient.

To select a slice (slab) at a specific location along z, an RF pulse must be sent with a bandwidth of frequencies that matches the range of frequencies in that slab, ensuring that the spins in middle slab are all on resonance with the excitation pulse and will absorb energy, changing from a low-to-high energy spin state. The thickness of the slice (Δz) can be controlled by varying the bandwidth ($\Delta\omega$) of the pulse or the strength of the gradient (G), as shown in **figure 6.6**.

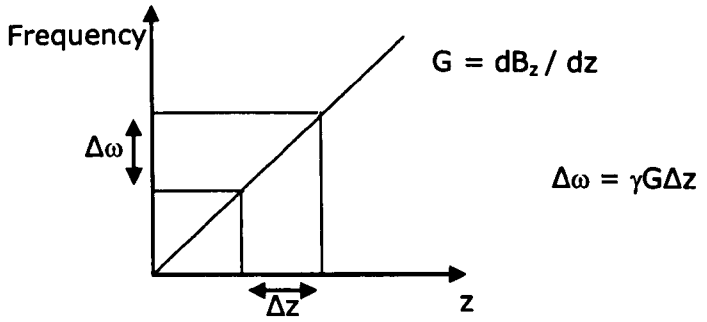

Figure 6.6: Schematic and corresponding equations to illustrate slice selection. The strength of the gradient G defines the slope of the line.

Immediately after the excitation pulse, the affected spins will undergo T_1 and T_2 relaxation processes giving rise to complete loss in MR signal, and so the sequence of gradients to apply in-plane encoding must be applied very quickly after slice-selection in order to resolve information about the distribution of nuclei within the slice.

6.1.2.2 Frequency and phase encoding

Frequency and phase encoding are intertwined processes used to cause spins at different spatial locations within the slice to precess at different rates and phases so that they can be measured and spatially encoded. Phase encoding is applied before the data acquisition period so that the spins accumulate phase offsets which are spatially dependent (e.g.: along x, in sagittal (Left/Right) plane). Sequential application of gradients (e.g. along y, in coronal (Anterior/Posterior)) within the slice alters the spin precession frequencies in a spatially controlled way in that direction. The application of this second gradient is called frequency encoding. For example, a strong positive phase encoding gradient would cause the spins at the left of the slice to accumulate different phase to those at the right. Once the spins along x have been varied, the

application of another gradient along y will alter the frequency of the spins and this is repeated. Phase and frequency encoding are repeated until all signal across the slice has been collected. Anatomical images are collected in this way but this can take tens of seconds/minutes. Therefore, this method is not ideal for fMRI, as images need to be acquired rapidly. The pulse sequence used for fMRI (EPI) rapidly acquires images on the order of milliseconds, and is described below.

6.1.2.3 EPI pulse sequence

K-space is the Fourier transform of image space. Converting k-space data into an image is known as image reconstruction. In the acquisition scheme described above each line of k-space is acquired following an excitation pulse which is time consuming. Echo planar imaging (EPI) uses fast switching of gradients to collect all lines of K-space after a single RF excitation. EPI is a fast and efficient encoding technique, acquiring an entire MR image in a fraction of a second. This sequence has the advantage of temporal resolution, but has coarser resolution compared to structural scans and so cannot be used for anatomical detail and diagnosis. Instead, this pulse sequence is fast enough to allow imaging of rapid physiological changes in the human body and is therefore used in functional MRI studies, with its contrast optimised to detect such changes. The following sections describe how the changes in brain physiology during sensory tasks can be utilised indirectly to collect data on brain function (cortical activation).

6.1.3 Introduction to fMRI and brain physiology

Brain activity is relatively localised. During sensory stimulation, such as tasting sugar, sensory neurons transmit sensory information from the taste receptor by generating action potentials. The area of the brain known as the taste cortex responds, which leads to an increase in metabolism in that area. This causes a large increase in oxygenated blood flow to rapidly deliver oxygen to that area, resulting in an increase in local blood oxygenation with brain activity. The MR signal is sensitive to the different magnetic susceptibilities of oxygenated and deoxygenated blood. Oxygenated blood is diamagnetic and has a similar magnetic susceptibility to tissue resulting in a slower T_2* decay. Deoxygenated blood is paramagnetic compared to tissue and therefore has a faster T_2* decay. This causes a local field gradient between the inside and outside of the vessels causing dephasing of spins and therefore different signal strengths which can be measured as a function of brain activity.

6.1.3.1 Brain metabolism and BOLD contrast

Approximately 20% of the body's oxygen and glucose is consumed by the brain where the vast majority is used for the maintenance of post-synaptic potentials. Blood oxygenation level dependant (BOLD) contrast relies on the magnetic properties of the haemoglobin molecule which are different depending on their oxygenation status. Deoxyhaemoglobin is strongly paramagnetic which means that the iron in the haemoglobin group is in a high-spin ferrous state and causes an increase in spin dephasing and therefore a decrease in T_2 relaxation times (faster T_2* decay). Conversely oxyhaemoglobin is diamagnetic and in a low-spin state so an increase in oxygenated blood to an active brain area would cause a decrease in the

proportion of deoxyhaemoglobin causing an increase in T_2 relaxation times (therefore slower T_2* decay). It is the difference between an area surrounded by oxyhaemoglobin (activated area) and an area surrounded by deoxyhaemoglobin (inactive area) which causes a local inhomogenous magnetic field distribution leading to a higher amplitude BOLD signal in the active brain tissue. The haemodynamic response function (HRF) is the time course of the BOLD signal change associated with neural activity. There are three key features of the HRF; the initial dip, the positive BOLD response peak and the post-stimulus undershoot, as shown in **figure 6.7**.

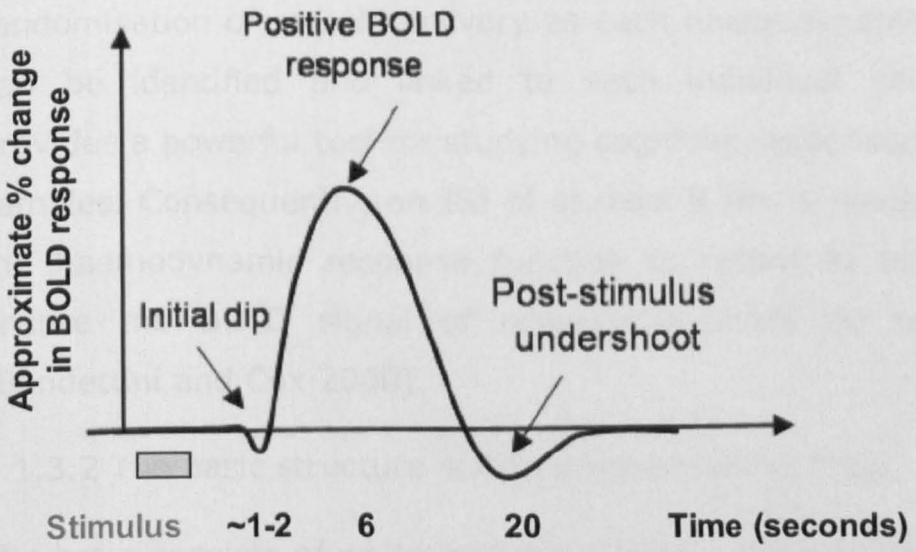

Figure 6.7: The haemodynamic response function (HRF) showing initial dip, positive BOLD response peak and post-stimulus undershoot after a stimulus.

The initial dip occurs due to the drop in venous oxygenation before the blood flow rise has an effect (immediately after stimulation) and can last 1-2 seconds. The positive BOLD signal peaks after approximately 6 seconds due to the time taken for oxygenated

blood to fill the capillaries and veins. The supply of oxygenated blood far exceeds consumption causing less spin dephasing, increased T2* and an increase in BOLD signal, which is of the order of 3% at 3T. It is the positive BOLD signal response that is typically studied in fMRI studies. The undershoot occurs after the end of stimulation due to an increase in deoxygenated blood leading to a reduction in BOLD signal due to stretching of venous vessels in response to increased blood flow.

The key features of the HRF are taken into account when designing fMRI experiments. This study used an event-related stimulus delivery design where a liquid sample was delivered as an isolated brief event (2 sec), separated in time from another. This allows randomisation of sample delivery as each haemodynamic response can be identified and linked to each individual sample. This provides a powerful tool for studying cognitive responses to specific samples. Consequently, an ISI of at least 8 sec is needed to allow the haemodynamic response function to return to baseline and ensure the BOLD signal of consecutive trials do not overlap (Bandettini and Cox 2000).

6.1.3.2 The basic structure and functioning of the brain

The brain consists of white and grey matter. White matter contains bundles of myelinated axons which appear white due to the fatty myelin sheath. Grey matter of the cerebral cortex contains the neuronal cell bodies and therefore appears grey. Grey and white matter have different MR physical properties which can be discriminated during MRI by using different weightings, as explained in the previous sections.

There are four main parts of the brain; the brainstem, cerebellum, cerebrum and the diencephalon as illustrated in **figure 6.8**.

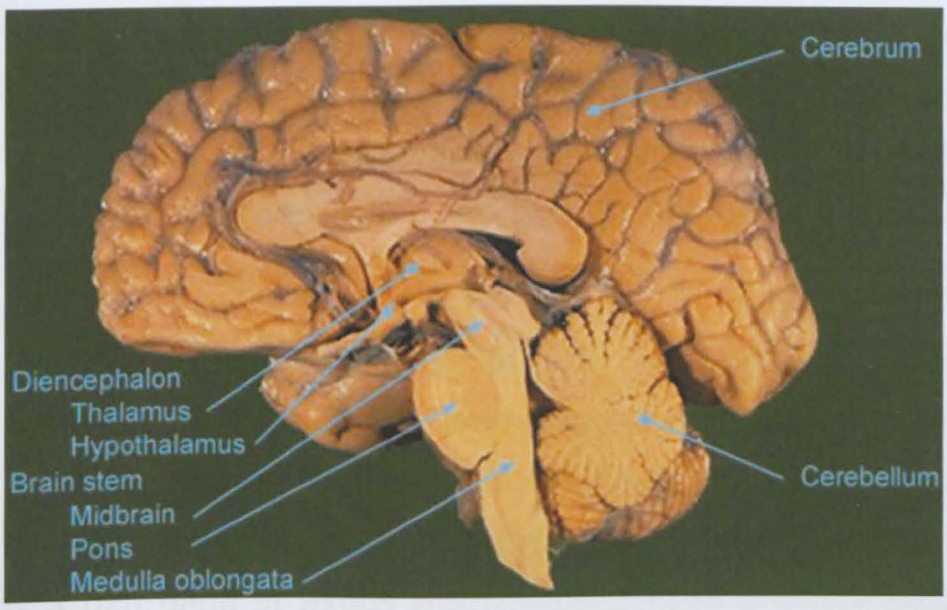

Figure 6.8: The basic structure of the brain. Source: http://www.colorado.edu/intphys/Class/IPHY3430-200/image/brainregions.jpg

The brainstem consists of the midbrain, medulla oblongata and pons. It extends out of the lower part of the brain and connects to the spinal cord providing a pathway for all fibre tracts to pass up and down from the peripheral nerves and spinal cord to the higher parts of the brain. Functions necessary for survival and arousal are located in the brainstem such as; breathing, digestion, heart rate, blood pressure, being awake and alert. The cerebellum sits behind the brainstem and helps to co-ordinate movement, balance and muscle co-ordination. The cerebrum contains the cerebral cortex, limbic system and basal ganglia. The limbic system is a set of brain structures responsible for actions relating to basic needs and emotions. The diencephalon consists of the thalamus and hypothalamus. The thalamus is comprised of the ventral posterior

medial (VPM) nucleus, the ventral posterior lateral (VPL) nucleus and the ventral posterior inferior (VPI) nucleus. It provides a gateway to the cerebral cortex as sensory (except olfactory) and motor activities are relayed and integrated within the thalamus before being projected to the sensory areas of the cortex. The primary function of the hypothalamus is to maintain homeostasis and general body metabolism. The lateral part is responsible for the control of food intake and satiety. The hypothalamus also plays a role in memory and awareness.

The brain cortex is divided into two hemispheres, left and right. Information entering the brain crosses over and consequently the right hemisphere controls the left-hand side of the body and the left-hemisphere controls the right-hand side. The corpus callosum is a rich band of axons which project from one hemisphere to the other. Each hemisphere is split into four main lobes; the frontal lobe, the occipital lobe, the parietal lobe and the temporal lobe as shown in **figure 6.9**.

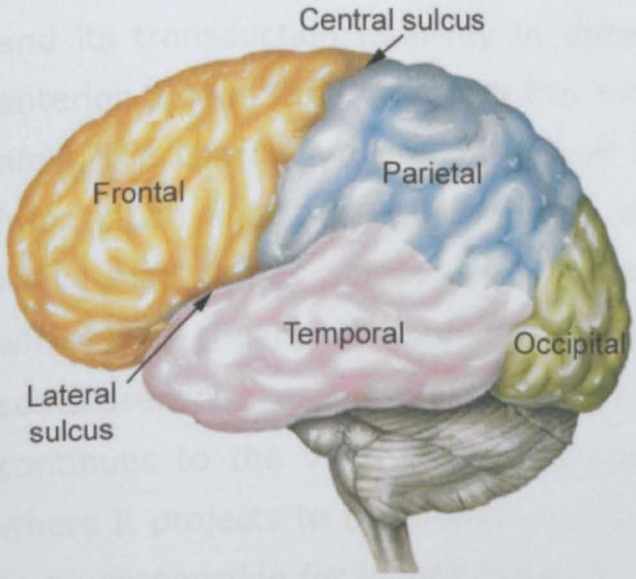

Figure 6.9: The four lobes of the brain, the frontal lobe, occipital lobe, parietal lobe and temporal lobe. Source: http://www.colorado.edu/intphys/Class/IPHY3730/image/figure5-22.jpg

The frontal lobe is involved in planning, organising, problem solving, selective attention and personality. The anterior portion is called the prefrontal cortex and is important for 'higher' cognitive functions including behaviour and emotions. The posterior portion (the post-frontal cortex) consists of premotor and motor areas. The occipital lobe processes all types of visual information, from reception of visual stimuli to visual recognition of shapes and colours. The parietal lobe contains the primary and secondary somatosensory cortices which control sensation (touch, pressure, pain and temperature) and judgement of fine sensation such as texture, weight, size and shape. The temporal lobe is involved in distinguishing sound and smell. The right temporal lobe is involved in visual memory (pictures and faces), while the left lobe is involved in verbal memory (words and names).

6.1.4 Cortical representation of taste

Section 1.1 in chapter 1 describes the peripheral gustatory system and its transduction pathway in detail. Taste activation from the anterior tongue (and therefore the fungiform papillae) is projected along the chorda tympani branch of the facial nerve transmitting information about the taste quality and quantity (intensity). This nerve also carries somatosensory afferents which (in association with the trigeminal nerve) provide information about the somatosensory aspects of the stimulus. The central pathway continues to the ventral-posterior-medial (VPM) of the thalamus where it projects to the primary gustatory cortex which is thought to be responsible for identifying taste quality. A number of studies have investigated the cortical representation of taste with the primary gustatory cortex being identified as the anterior insula,

frontal operculum (Kobayakawa, Endo et al. 1996) and postcentral gyrus. Taste projections may then continue to the amygdala, orbito-frontal cortex (OFC) (the secondary taste cortex) and anterior cingulate cortex (ACC). The OFC and the ACC have been associated with afferent aspects of taste (Francis, Rolls et al. 1999). Therefore the main brain areas expected to be activated during gustatory stimulation are; thalamus, insula cortex, amygdala, ACC and the OFC. The anatomy of each is discussed below.

The thalamus, situated in the cerebellum at the top of the brainstem, is considered the gateway to the cerebral cortex because all sensory (except olfaction) and motor activities are projected here before being received by their respective sensory areas. The insula lay deep beneath the frontal, parietal and temporal opercula surrounded by a sulcus that separates it from the frontal, parietal and temporal lobes. It is subdivided into three parts; anterior insula, mid-insula and the posterior insula. The amygdala is a major component of the limbic system playing an important role in the pleasure (Zald, Lee et al. 1998; O'Doherty, Rolls et al. 2001) and the intensity of food (Small, Gregory et al. 2003). The cingulate cortex is divided into anterior (ACC) and posterior (PCC) portions. The ACC has been shown to have a 'hedonic' response to afferent aspects of food (Rolls; Zald, Lee et al. 1998; Francis, Rolls et al. 1999; de Araujo and Rolls 2004; Grabenhorst, Rolls et al. 2007; Grabenhorst, Rolls et al. 2010). The OFC is part of the ventral surface of the prefrontal cortex. It receives input from gustatory, olfactory, somatosensory, visual and auditory simulation and for this reason is considered a 'multimodal' area (Rolls and Baylis 1994; Kadohisa, Rolls et al. 2004; Verhagen and Engelen 2006; Rolls, Critchley et al. 2010). The OFC is termed the secondary taste cortex (Baylis, Rolls et al. 1995) because it has

been discovered to act as a higher order taste centre involved in emotional values of taste (Heinzel and Northoff 2009). For example, the OFC has been found to respond to hunger but not satiety (Haase, Cerf-Ducastel et al. 2009). However, subjects will not be in a fasted state of hunger during scanning and therefore the potential activation in the OFC in this study is unknown.

6.1.5 Cortical representation of trigeminal stimuli

The oral trigeminal system and the processing of somatosensory (tactile, temperature and pain) stimuli experienced during food and beverage consumption was reviewed in chapter 1, section 1.3. CO_2 dissolved in beverages elicits two somatosensory receptors; the 'tingle' of CO_2 is sensed by the nociceptors which detect pain, and the tactile aspect of the bubbles is detected by the mechanoreceptors. The pathway of both receptors leads ultimately to the primary (SI) and secondary (SII) somatosensory cortices, located in the postcentral gyrus which process somatosensory information from the body. The surface of the body is topographically represented in the primary somatosensory cortex in a manner dependent on the density of the neurons. Neuron density is greatest in the oral cavity (including lips) and hands, as illustrated by the somatosensory homunculus (**figure 6.10**). Somatosensory activation in the oral cavity (pharynx, tongue, jaw and lips) is represented in the most ventral part of SI (Tamura, Shibukawa et al. 2008), lateral to SII.

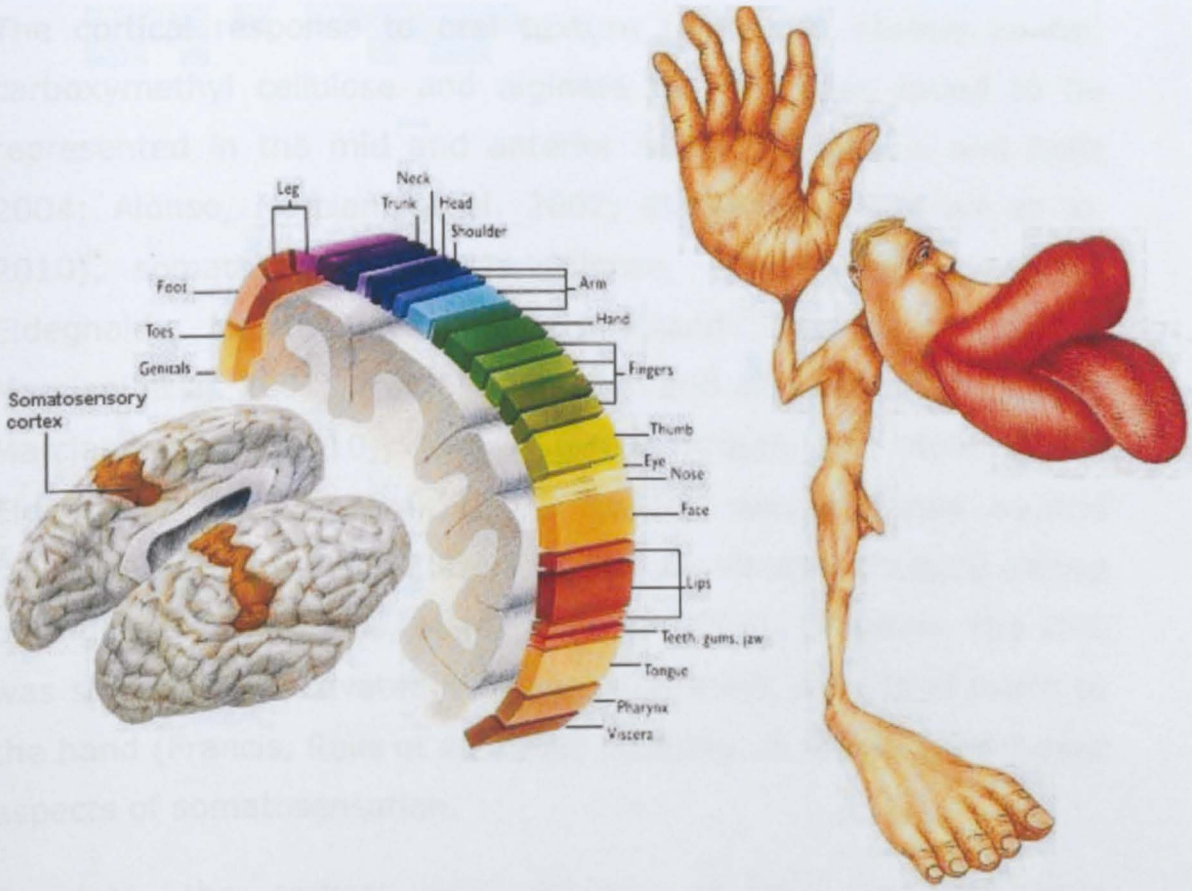

Figure 6.10: The somatosensory homunculus, illustrating the representation of the oral cavity in the most ventral part of the primary somatosensory cortex. Source:http://www.amareway.org/wp-content/uploads/2010/07/homunculus-somatosensory-cortex-universe-review-ca.jpg

SII receives input mainly from SI and directly from the thalamus. It projects back to SI, the primary motor cortex and the posterior insula (Youell, Wise et al. 2004). SII, (along with somatosensory association areas in the superior/posterior parietal lobe), is thought to be involved in tactile discrimination (Maldjian, Gottschalk et al. 1999) while SI is related to the physical aspects (Francis, Rolls et al. 1999; Sakamoto, Nakata et al. 2008). Few studies have investigated the cortical response to oral somatosensation and no studies have investigated the response to CO_2. The majority of the literature investigating somatosensation is cutaneous.

The cortical response to oral texture (viscosity) elicited by fat, carboxymethyl cellulose and alginate gel has been found to be represented in the mid and anterior insula (de Araujo and Rolls 2004; Alonso, Marciani et al. 2007; Eldeghaidy, Marciani et al. 2010), somatosensory cortex (Alonso, Marciani et al. 2007; Eldeghaidy, Marciani et al. 2010), rolandic operculum (Alonso, Marciani et al. 2007), frontal operculum and amygdala (Eldeghaidy, Marciani et al. 2010), and ACC (de Araujo and Rolls 2004; Eldeghaidy, Marciani et al. 2010). The OFC was activated by oral fat (de Araujo and Rolls 2004) but not by viscosity/texture elicted by alginate gel (Alonso, Marciani et al. 2007). However, the OFC was significantly activated by pleasant afferent aspects of touch to the hand (Francis, Rolls et al. 1999) implying an role in the afferent aspects of somatosensation.

To date, the cortical representation of pain has not been investigated in the oral cavity. A study investigating pain from a CO_2 laser on the calf showed activation in SI, SII, insula cortex and thalamus, with SI and the thalamus being proposed to be involved in the sensory discriminative components of pain (Youell, Wise et al. 2004).

Oral temperature was found to activate the insula, somatosensory cortex, OFC, ACC and the ventral striatum (Guest, Grabenhorst et al. 2007). Areas correlated to the pleasantness of oral temperature ratings included the OFC and ACC. Given that both these areas have been found to respond to pleasantness of other sensory stimuli, it seems likely that the OFC and ACC could act as multimodal afferent areas. Furthermore, activation in the OFC and ACC during thermal stimulation to the hand was correlated with unpleasant thermal stimuli (Rolls, Grabenhorst et al. 2008). In the

same study, SI and the posterior insula were correlated with intensity but not pleasantness of thermal stimuli (Rolls, Grabenhorst et al. 2008). Therefore the cortical areas that respond to the affective value of thermal stimuli seem to be different to those that respond to the intensity.

6.1.6 Effect of taster status on cortical activity

Taster status, as reviewed and investigated in the previous chapter seems to impact on population differences in taste perception. To date, only one study, which was also within our faculty, has investigated the cortical response between PROP taster groups using different fat levels in liquid emulsions as the stimuli. Results showed a strong correlation of PROP taster status with cortical response in the somatosensory areas supporting the theory that super-tasters have greater somatosensory acuity (Eldeghaidy, Marciani et al. 2010). Fourteen subjects took part in the study (5 x PROP non-tasters, 4 x PROP medium-tasters and 5 x super-tasters) which allowed a correlation with PROP intensity to be assessed. Whilst the combined group of 14 subjects is sufficient for a group analysis of cortical activation correlated to fat, the differences between PROP taster groups requires at least 10 subjects in each group. The current study took a controlled approach to determine the difference in cortical response between PROP taster groups (PROP non-tasters, PROP medium-tasters and PROP super-tasters) and thermal taster groups (thermal tasters and thermal non-tasters).

6.1.7 Data analysis

Non-task related signal variability such as thermal noise, system noise, physiological noise and noise from head movement must be minimised or eliminated during the pre-processing steps of data-analysis. This increases the functional signal-to-noise ratio (SNR) which is important for statistical analysis to identify which voxels in the images show a hameodynamic response to the sample. It must be noted that the haemodynamic response is small, typically of less than 3 % at 3 T, and thus a number of trials of stimulation (replicates) are typically performed. An overview of the fMRI data analysis steps is illustrated by **figure 6.11** and is explained in the following bullet points.

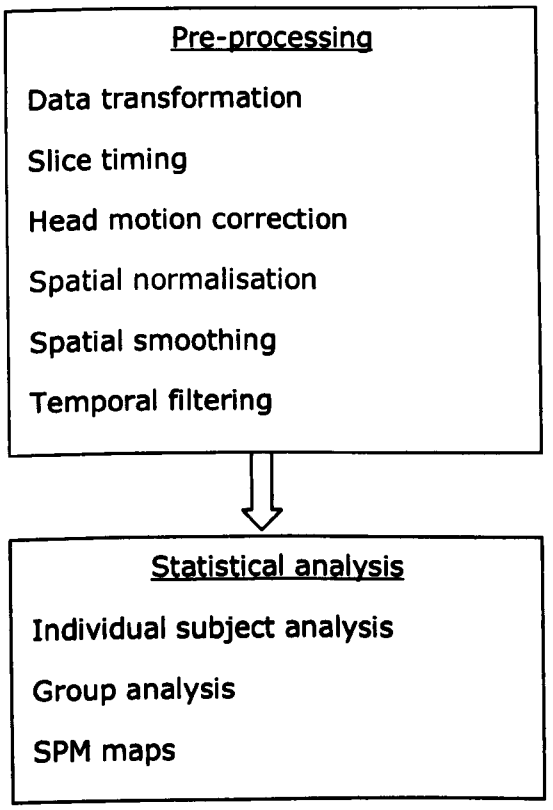

Figure 6.11: An overview of the fMRI data analysis

- Data transformation: Data is first transformed from K-space into image space.

- Slice timing correction: Slices are acquired at slightly different times which can cause a phase shift in the BOLD haemodynamic response. Therefore slice timing correction shifts each voxel's time series (within a slice) to a reference slice so that all voxels in the same volume appear to be acquired at the same time.

- Head motion correction: small head movements during scanning can lead to a change in the same voxel's location over the fMRI time series. Motion correction eliminates the extent of the head motion across the time series by comparing it to a reference image using a 6-parameter rigid body correction. All images are then realigned to the reference image. The transformations should be scrutinised for excessive motion. Any subject moving more than one voxel should be removed from the study.

- Spatial normalisation: In fMRI studies, data is usually collected from a number of subjects (typically 10-12) and a group map is formed. However, individual brains differ in shape, size and orientation so the data from each subject is transformed to a standard 'template' space so that group activation maps can be formed. The most commonly used template spaces are the Montreal Neurological Institute (MNI space) and Talairach space. MNI space was used in this study.

- Spatial smoothing: Spatial normalisation does not solve the problem that subjects have different cortical organisation. Spatial smoothing must therefore be applied to allow group

analysis of data to take place. This increases the likelihood of overlapping activity across subjects by blurring the sharp edges of the image which removes high spatial frequency noise and consequently enhances SNR.

- Temporal filtering: A high pass temporal filter is applied to remove unwanted slow varying signals in the fMRI time series such as, scanner drift and physiological noise (respiration and cardiac pulsation).

After the pre-processing steps are complete, each of the individual subject's data sets can be subjected to statistical analysis. The general linear model (GLM) is the most common way to analyse fMRI data with many dependant variables. It assumes that the BOLD responses can be linearly modelled by convolving the stimulus waveform with the haemodynamic response function. The experimental design is represented by a data matrix consisting of rows of time points (volumes) in the BOLD time series and columns of experimental stimuli/samples and confounds such as the estimated motion parameters. Noise is independently distributed across voxels and the resulting data is able to reliably identify regions showing a significant experimental effect of interest.

In fMRI, there are two main forms of group statistical analysis, fixed effects analysis (FFX) and random effects analysis (RFX). FFX assumes that the experiment effect is constant and all subjects are affected similarly by the stimulus, thereby only taking into account the within subject variability. Conclusions can therefore only be made about those particular subjects in the group using FFX. RFX takes into account the variability across subjects as well as within subject variability. Therefore conclusions about the population (from which the subjects represent) can be made using this

method. In RFX, a design matrix and statistical analysis are performed on each individual subject, (producing a statistical map for each subject in each sample/condition). The statistical map from each subject is then pooled for second level RFX analysis to give a combined statistical map for the group (for the sample/condition selected). The significantly activated areas which overlap when the individual subject statistical maps are combined at group level are shown as the statistically active areas. A parametric test (T- or F- statistic) is carried out to test the statistical significance of the activated voxels on a voxel-by-voxel basis. The resulting T or F values are converted to z-scores and probability (P) maps. The statistical parametric maps (SPMs) are colour coded to show significant activation and superimposed onto anatomical T_1 weighted images such as a MNI template. Data can be displayed at uncorrected probability (typically $p<0.001$) or a corrected probability (FWE or FDR) typically $p<0.05$, which corrects for false positives. Family wise error (FWE) rate controls the chance of any false positives using random theory and is more conservative than the false discovery rate (FDR) which is less strict and controls the fraction of false positives.

The resulting data shows areas of the brain significantly activated in response to the sample or stimulus, and brain structures can be correlated to certain functions. The objectives of this study were to; investigate the cortical activation to sweet taste as elicited by dextrose and the effects of oral trigeminal stimulation elicited by carbon dioxide (CO_2); compare the differences in cortical activation between population groups with different oral sensitivity.

6.2 MATERIALS AND METHODS

6.2.1 Subjects

Procedures were conducted with University of Nottingham Medical School ethics committee approval and written consent was obtained from all subjects. Subjects were asked to complete an MR scanner safety screening questionnaire (appendix 1) prior to the scan session. Only those subjects who were considered 'MR safe' were invited to take part in the study. From the 52 subjects screened for PROP and thermal taster status in Chapter 5, 36 subjects (24 females, 12 males) were invited to take part in this study comprising 12 PROP non-tasters (pNT's) (7 females, 5 males), 12 PROP medium-tasters (pMT's) (8 female, 4 male) and 12 PROP super-tasters (pST's) (9 female, 3 male). Twelve subjects were classified as thermal tasters (TT) (8 females, 4 males) and the first 12 of the remaining 24 thermal non-tasters (TnTs) scanned were selected as the TnT group (7 females, 5 males). All 36 subjects took part in the fMRI scan session. Subjects were in the scanner for approximately 1 hour. The whole session lasted approximately 2 hours including study explanation and behavioural assessments. A disturbance allowance was paid to those subjects who participated.

6.2.2 Stimuli

Three samples, differing only in their CO_2 level were prepared by dissolving 70g/L of polydextrose (Litesse® Ultra powder, Danisco Sweeteners, New Century, KS, USA) and 30g/L of dextrose (MyProtein, Manchester, UK) into mineral water (Danone, Paris, France) and mixed on a roller bed for 6h to ensure full dispersion. Samples were refrigerated until they reached 6°C (±1). The

carbonation apparatus and method have been previously described in Chapter 2 and will be briefly explained here. Samples to be carbonated were aliquoted into 100ml Schott bottles (Fisher Scientific, Loughborough, UK) fitted with modified Schott bottle caps (Fisher Scientific, Loughborough, UK). The caps were modified in house (Medical Engineering Unit, University of Nottingham, UK) to allow a one-way flow of food grade CO_2 (BOC, Guildford, UK) directly into the vessel ensuing accurate carbonation levels. The low CO_2 samples were carbonated to 1 volume and the high CO_2 samples to 2 volumes. The sample was disconnected from the carbonation equipment and stored at 5°C ± 1 until required.

6.2.3 Preference data

Prior to the fMRI scan, subjects were presented with three 40ml coded samples; no CO_2, low CO_2 and high CO_2 and were asked to taste them and place them in order of preference. After this task, subjects were told what the samples were (no, low and high CO_2) and that these were examples of the solutions that would be delivered into their mouths in the MR scanner. A full explanation of the fMRI session was then given.

6.2.4 Stimulus delivery

Previous studies investigating the cortical response to taste using fMRI techniques have used automated pump systems for liquid sample delivery. The advantages of this system are strict control over the time, intensity/force and volume of sample delivery. However, the pump system contains metal components and must be placed a fixed distance away from the magnet for safety. The

safe delivery of liquids using this method therefore requires long lengths of the tubing (typically 20m) to be fed through from the system to the subject in the scanner. During experimentation with this method which uses long tubes, it was found that the CO_2 was unstable in this length of tubing resulting in uncontrolled loss of CO_2 from the liquid during the scanning period. In addition, the pumps were not strong enough to pump a pressurised liquid CO_2 system. For this reason a manual delivery system was chosen as much shorter lengths of tubing (2m) could be used, restricting CO_2 loss over time.

Four 60ml syringes (BD, Oxford, UK) were filled with the three stimuli (No CO_2, low CO_2 and high CO_2) and Evian mineral water (Danone, Paris, France). Each syringe had a stopcock attached by leur lock fittings to prevent the loss of CO_2 from the syringes and to control flow of the sample, as shown in **figure 6.12**. Plastic tubing, 68cm long with 1.5mm bore width was connected via leur lock fittings to the leur stopcock at one end and fixed into a bite bar at the other end.

Figure 6.12: Syringe, stopcock and sample delivery tubing. The stopcock is connected to the syringe and delivery tubing is connected to the stopcock.

For each individual subject, a unique bite bar was created out of dental putty (UnoDent, Essex, UK) on the day of the scan session.

The four delivery tubes were sandwiched centrally between two pieces of dental putty and the subjects were instructed to place the putty in their mouth and gently bite down until they felt the tubes between their teeth. This procedure was very similar to creating a dental impression except the resulting bite bar had 4 tubes protruding through the centre for sample delivery onto the tongue, as illustrated by **figure 6.13**.

Figure 6.13: Dental impression made into a bite bar to secure the sample delivery tubes.

The purpose of the bite bar was to keep the location of the tubes in a fixed position in the mouth to ensure stimulus delivery onto the same place on the tongue with each replicate and across blocks. It also aided reduction of head movement during scanning. Dead space was removed from the tubes by delivering 5ml of the sample into the subject's mouth before commencing scanning. Samples were manually delivered according to a randomised design, fixed across blocks and subjects.

6.2.5 fMRI paradigm

One cycle of the fMRI paradigm is shown schematically in **figure 6.14**. In each cycle, 2 mL of sample were manually delivered over a 2 sec period (flow rate 1 mL/sec) into the subjects mouth via the

delivery tubes fixed in the subject's bite bar. Samples differing in carbonation level were delivered in a random order, the order of which was fixed in each block. Each block consisted of 10 x No CO_2, 10 x low CO_2 and 10x high CO_2 samples. Subjects were cued to swallow after the sample was delivered by a visual cue (small cross) presented immediately after each stimulus delivery using Presentation Software (Neurobehavioral System, San Fransisco, US). Subjects could see the cue via a mirror attached to the head coil. To minimise visual stimulation from the visual cue, the cross was presented throughout the paradigm and altered from white to purple to instruct subjects to swallow. All subjects were provided with a MR compatible button box with three buttons. On the cue following swallowing, subjects were asked to press a button to identify the level of carbonation in the sample received, 1 = No CO_2, 2 = low CO_2 and 3 = high CO_2. The responses were collected in the Presentation log file. Following the delivery of each stimulus, 1ml of Evian mineral water (Danone, Paris, France) was delivered over a 1 sec period to clear the oral cavity of any lingering stimulus (water wash). All stimuli and the water wash were delivered at 6°C ±1. A delay of 12 s was given between the stimulus delivery and the water wash to allow for data acquisition and the HRF to return to baseline. A delay of 7.5 s was allowed after the water wash before repeating the cycle.

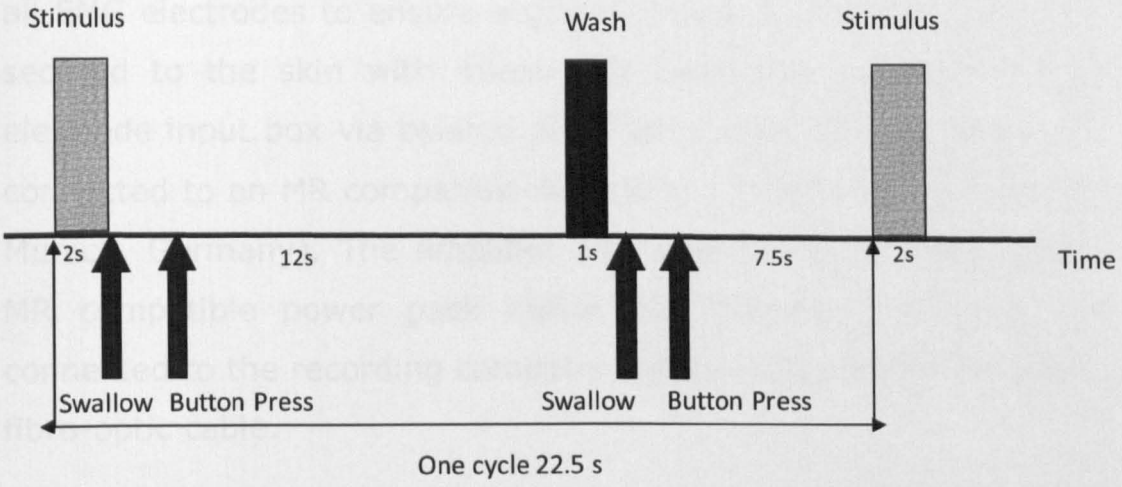

Figure 6.14: One cycle of the fMRI paradigm

For each subject, 30 cycles (10 of each stimulus in a randomised order) were delivered over 1 block. Each block took just over 11 minutes to complete. Syringes were refilled with fresh samples during a short break (<5mins) before the next block commenced. 3 blocks were acquired in each fMRI session, collecting a total of 30 replicates for each sample for each subject.

6.2.6 EMG

Subjects were given a visual cue to indicate when to swallow following stimulus delivery. However, it is difficult to swallow on cue and so the exact time of swallowing was monitored by surface Electromyography (EMG). A pair of MRI compatible Ag/AgCl electrodes were placed over the suprahyoid (swallowing) muscles to the left and right of the laryngeal prominence (Adam's apple). Two further electrodes served as ground and reference points and were placed on the mastoid part of the temporal bone behind the ear (reference) and on the boniest part of the clavicle/acromion shoulder bones (ground). Conductive electrolyte gel was applied to

all EMG electrodes to ensure a good contact. Electrodes were then secured to the skin with micro-pore tape and connected to an electrode input box via twisted electrode leads. The input box was connected to an MR compatible BrainAmp amplifier (BrainProducts, Munich, Germany). The amplifier was powered by a rechargeable MR compatible power pack inside the scanner room and was connected to the recording computer outside the scanner through a fibro-optic cable.

The Vision Recorder software (BrainProducts, Munich, Germany) was used to record the EMG signal. Data were collected with a sampling frequency of 5 kHz and band-pass filtered at 0.016 - 250 Hz. An in-house frequency divider was used to synchronise the MR scanner clock and EMG clock. The frequency divider used a 10 MHz signal exported from MR scanner and converted this to a 5 kHz signal for the EMG system. The divider was connected to the EMG system through the USB interface box, which was also connected to the presentation computer in order to detect the scanner and stimuli triggers. The MR scanner delivered a marker to the Brain Vision Recorder at the time of each fMRI volume acquisition, allowing a template for MR artefact correction to be formed (Allen, Polizzi et al. 1998; Allen, Josephs et al. 2000). Artefacts commonly arise from simultaneous EMG recording during fMRI acquisition due to subject movement, movement of EMG electrodes and leads and variation in magnetic field and the RF pulse sequence during image acquisition. Artefacts can be minimised by asking the subject to remain still, twisting the EMG leads and applying a low pass filter to the data.

EMG data were processed using Brain Vision Analyzer (v2.0) software. Data was first corrected for MR artefacts, down-sampled

to 500 Hz, and a 70 Hz low-pass filter was applied to remove the residual artefacts. A bipolar derivation was then calculated for the two recorded channels and a band-pass filter of 0.5 to 30 Hz applied. **Figure 6.15** illustrates an EMG trace after artefact correction. This information was subsequently used in the fMRI design matrix to define the time the stimulus remained in the mouth to improve modelling of the fMRI data.

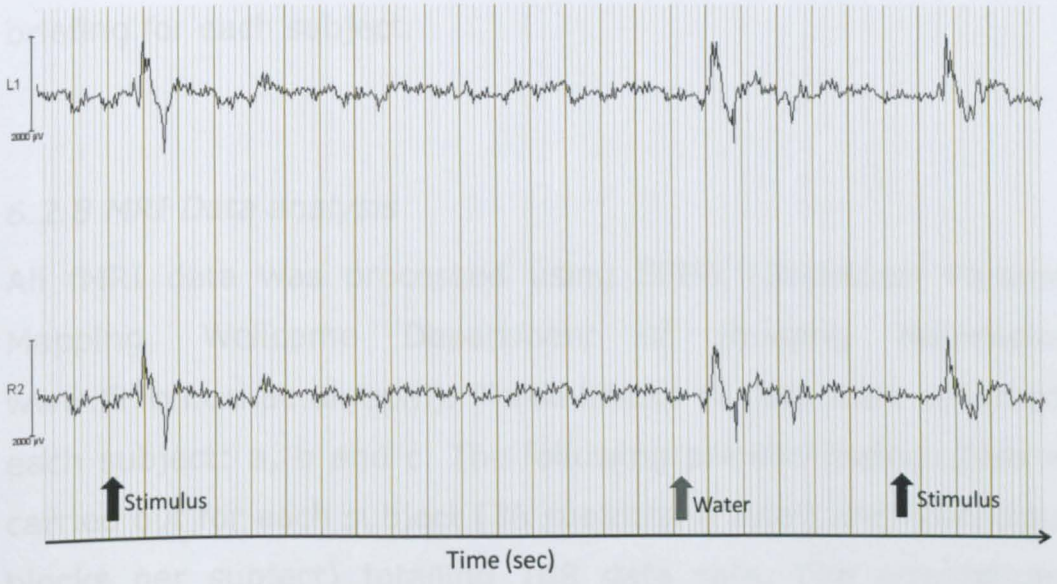

Figure 6.15: Artefact corrected EMG traces from both the right and left electrodes placed on the supahyoid (swallowing) muscles. Arrows show stimuli triggers.

6.2.7 MRI data acquisition

This study was performed on the 3 T Philips Achieva scanner with a 32 channel head coil. fMRI acquisition comprised of 3 x blocks of 34 transverse double-echo (echo times (TE) 20 and 45 ms) gradient echo-planar imaging (EPI) slices with 80 x 80 matrix and 3mm isotropic resolution. A double-echo acquisition was chosen to provide increased BOLD sensitivity in areas with a short transverse relaxation time (T_2*), such as the orbitofrontal cortex (Posse, Wiese

et al. 1999; Marciani, Pfeiffer et al. 2006; Gowland and Bowtell 2007)

The volume repetition time (TR) was 2.5 s (jittered). Padding was used where necessary to minimise head movements. A T_1 weighted MPRAGE anatomical image comprising of 160 slices with 256 x 256 matrix and 1mm isotropic resolution was acquired. Each MRI session lasted approximately 2 hours including scanner set-up and briefing for each subject.

6.2.8 MRI Data analysis

All fMRI data was processed using SPM5 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience; www.fil.ion.ucl.ac.uk/spm). Three blocks of data were collected for each subject: a, b and c. The following pre-processing steps were carried out for each subject (36 subjects in total) and each block (3 blocks per subject) totalling 108 data sets. The orientations of images from both echo time data sets were set to the AC-PC point. The first echo images were then realigned to correct for motion and the realignment transforms applied to the second echo data sets. Each realignment plot was individually visually inspected for excess motion (greater than a voxel) during each acquisition block. A weighted summation (Posse, Wiese et al. 1999) of the double echo fMRI data set was then performed based on the average T_2* in each voxel, as determined by averaging across the fMRI data set for each echo data set. Each first echo data set was then normalised to a standard template in MNI space, and the weighted data then moved to this space. The weighted data set was then smoothed with an 8-mm full width half-maximum (FWHM) isotropic

Gaussian kernel to improve the signal-to-noise ratio and to account for anatomical differences between subjects in the group analysis.

After pre-processing was complete, a general linear model was formed for each subject which would combine sample replicates across blocks to give one individual map of activated areas for each sample (no CO_2, low CO_2 and high CO_2), and all samples combined (all) representing the average response to CO_2 and taste combined. Convolution of the haemodynamic response function (HDF) with the stimulus onset and the time of swallow (from the EMG traces) were carried out for each individual and each block. The water wash and the button press onset were included in the design matrix as covariates of no interest. The individual subject-stimuli maps were then combined at a random effects (RFX) group level to assess the difference in brain activity between PROP and thermal population groups (group analysis). These SPM maps were then subjected to the following statistical analysis.

A one-sample t-test was created for each sample and all samples combined resulting in 4 group maps (p FDR< 0.05) for each stimulus (No CO_2, low CO_2, high CO_2 and 'all') in each population group. For example; 4 group maps for PROP non-tasters (pNTs), 4 group maps for PROP medium-tasters (pMTs) and so on. Significant activity was shown at FDR <0.05. In a second step, a linear (1^{st} order) parametric modulation using CO_2 concentration as the modulation parameter was adopted to identify areas of the brain showing haemodynamic response that increased (positive correlation) or decreased (negative correlation) linearly with CO_2 concentration. An explicit mask of all attributes of oral perception ($p<0.05$) was applied as *a priori* areas of interest and significant activity was shown at $P<0.001$ uncorrected. A 2-sample t-test was

carried out between groups for each sample to determine any significant difference in strength of activation (BOLD response) between the groups ($P< 0.01$ uncorrected). Paired t-tests were carried out within groups to determine which samples (no CO_2, low CO_2, high CO_2) significantly differed in their activation ($P<0.01$ uncorrected).

6.2.8.1 Region of Interest (ROI) analysis

The variability of BOLD activations within and across the different taste phenotype groups was assessed by calculating the activation strength (T-scores) in 7 brain regions of interest (ROIs). The ROIs were anatomically defined by the WFU PickAtlas tool in SPM5 and a mask was created for each region and hemisphere. The following masks were created;

- SI (mouth) defined as an 8mm sphere centred at 60, -6, 20
- SII defined as Brodmann area (BA) 43 dilated by 1
- Insula which was sub-divided into anterior-insula defined by an 8m sphere centred at 40, 10, -2; mid-insula defined as an 8m sphere centred at 40, 0, 0; posterior insula defined by an 8mm sphere, dilated by 1 centred at 44, -32, 12
- Thalamus, determined anatomically (aal)
- ACC defined by a 14mm sphere, dilated by 1 and centred at 2, -10, 56
- Amygdala, determined anatomically (aal)

- OFC which was subdivided into lateral-OFC defined as an 8mm sphere centred at 28, 30, -10 and medial-OFC defined as an 8mm sphere centred at 6, 44, -2.

T-scores from each ROI were interrogated for each group and each stimulus using an in-house program written by Dr. Susan Francis.

6.2.8.2 Behavioural data

Behavioural data of discrimination of CO_2 level was analysed by calculating the percentage number of correctly identified stimuli for each subject and associated d' values (Ennis 1993). D' is a measure of sensitivity representing probability of correct responses for that group. A value over 1 represents an ability to discriminate (Lawless and Heymann 2010). Significant differences between d' values between groups were calculated using the student t-test.

Samples were ranked by each subject for preference, ranging from most preferred to least preferred. The quantity of subjects who most and least preferred each sample was determined in each group and was compared against the other groups.

6.3 RESULTS

No data had to be discarded due to excessive motion as all realignment plots were within one voxel.

Thirty six subjects (12 males, 24 females) took part in the scanning sessions of which 12 (5 males, 7 females) were PROP non-tasters (pNTs), 12 (4 males, 8 females) were medium tasters (pMTs) and 12 (3 males, 9 females) were super-tasters (pSTs). Of the 36 subjects, a total of 24 (12 thermal tasters (TTs) and 12 thermal non-tasters (TnTs)) formed the thermal taster groups. The

remaining 12 subjects were TnTs but only the first 12 of that group to be scanned were selected to form the TnT group analysis. This ensures a balanced design for data analysis so that group comparisons can be made. In the TT group (4 males, 8 females), 4 were pNTs, 6 were pMTs and 2 were pSTs. In the TnT group (5 males, 7 females), 4 were pNTs, 6 were pMTs and 2 were pSTs. **Table 6.1** shows a summary of the subject's gender and their taster status (PROP or thermal) group classifications.

Table 6.1: Summary table of subject's gender and taster status group classifications. pNT = PROP non-tasters, pMTs = PROP medium-tasters, pST = PROP super-tasters, TT = thermal tasters and TnT = thermal non-tasters

Gender	PROP taster status			Thermal taster status		
Male	12 (33.33%)	pNT	5	9 (37.5%)	TT	2
					TnT	2
		pMT	4		TT	1
					TnT	3
		pST	3		TT	1
					TnT	0
Female	24 (66.66%)	pNT	7	15 (62.5%)	TT	2
					TnT	2
		pMT	8		TT	5
					TnT	3
		pST	9		TT	1
					TnT	2

The results from the behavioural and fMRI data will be presented and compared between taste phenotype groups below. During analysis of each group, BOLD activation strength to each sample was compared to determine the effects of CO_2 on taste activation. The anterior insula is part of the primary taste cortex. BOLD cortical activation strength was not significantly modified ($p>0.01$ uncorrected) in this region of interest due to CO_2 addition. Correlation analysis of the effect of CO_2 on BOLD activation showed positive linear increases in the somatosensory cortex with increasing CO_2 in most taste phenotype groups. This study is the first to show that CO_2 significantly increases activation in the somatosensory cortex.

6.3.1 Effect of PROP taster status on cortical activity

6.3.1.1 Sample preference

Figures 6.16 a, b and **c** show the preference of CO_2 as a percentage for each of the PROP taster groups; pNTs, pMTs and pSTs respectively.

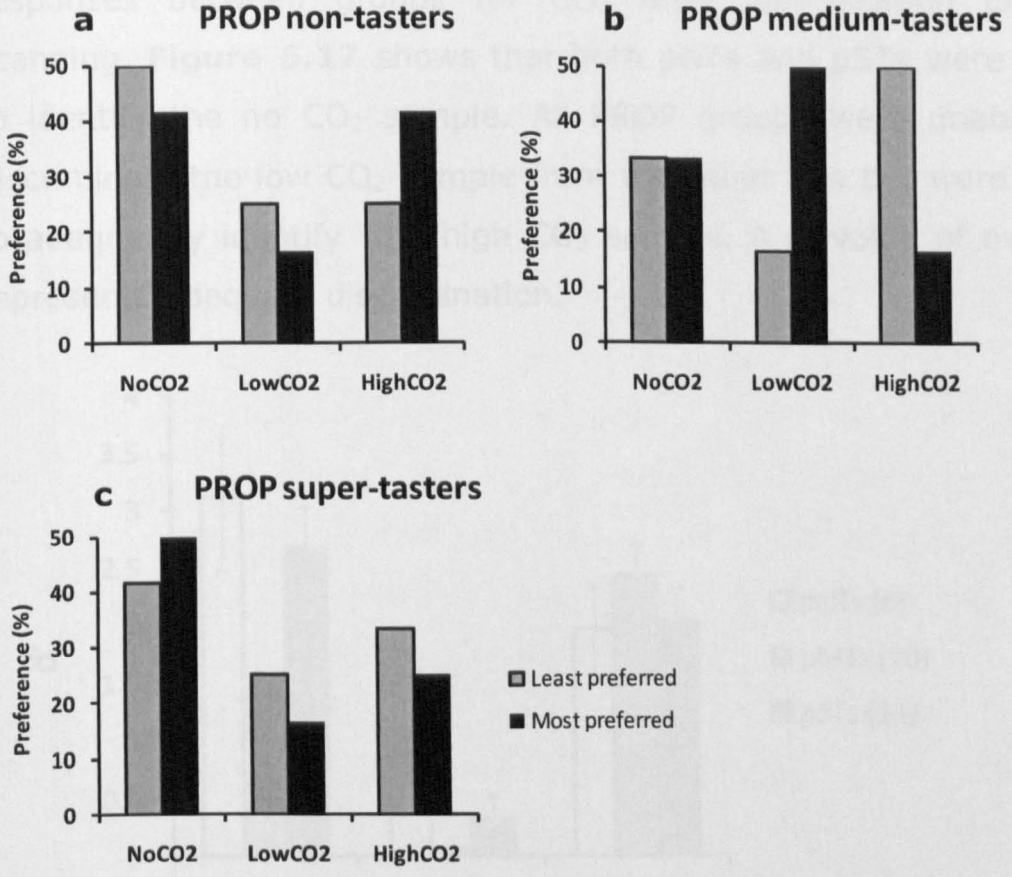

Figure 6.16: Percentage of subjects' sample preference in each PROP group. a = PROP non-tasters, b = PROP medium-tasters and c = PROP super-tasters. Grey bars show the least preferred sample and black bars show the most preferred sample.

PROP non-tasters did not seem to have a clear preference for any of the samples. PROP medium-tasters most preferred the low CO_2 sample and least preferred the high CO_2 sample, suggesting that CO_2 addition could drive preference in this group but must be at a low level in order to achieve optimum preference. PROP super-tasters did not show a clear preference for any of the samples.

6.3.1.2 CO_2 discrimination

Due to a computer malfunction, 6 pNTs, 2 pMTs and 1 of the pSTs button press responses were not saved resulting in unequal sets of

responses between groups for CO_2 level identification during scanning. **Figure 6.17** shows that both pNTs and pSTs were able to identify the no CO_2 sample. All PROP groups were unable to discriminate the low CO_2 sample from the other two but were able to adequately identify the high CO_2 sample. A d' value of over 1 represents adequate discrimination.

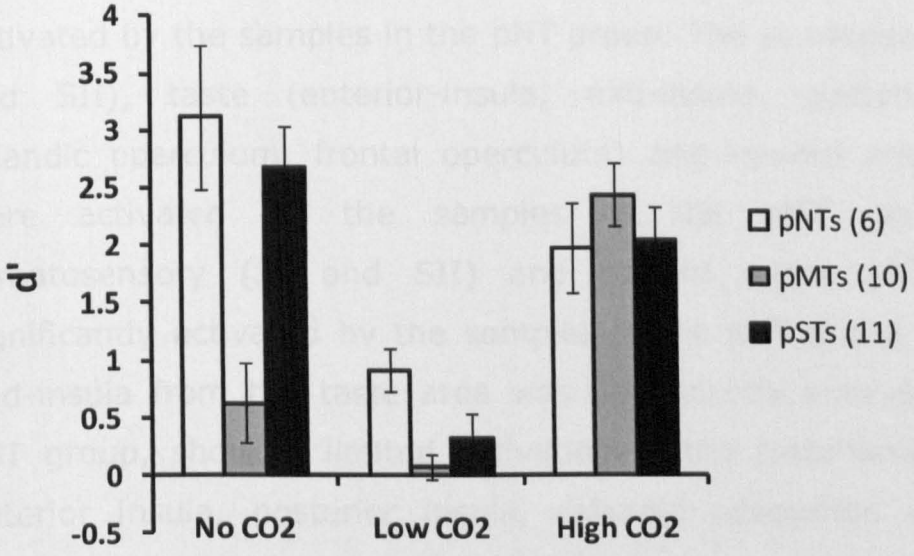

Figure 6.17: Identification of CO_2 level during scanning. Mean d' value of each group of subjects. Number of subjects from each group where data was collected is shown in parentheses.

6.3.1.3 Brain areas activated by the samples

The random effects group analysis (RFX) for each of the PROP groups revealed the areas of the brain significantly activated in response to 'all' (no CO_2 + low CO_2 + high CO_2) samples. A complete list of these brain areas, along with their location in MNI co-ordinates, T-scores and P value corrected for false discovery rate (FDR) <0.05 can be found in **appendix 2** for pNTs, pMTs and pSTs. A summary table of the brain areas significantly activated (FRD < 0.05) by each group is shown in **table 6.2**. Activation was

found in the somatosensory (SI and SII, and posterior insula,) and taste (anterior insula, mid-insula, posterior insula, rolandic operculum and frontal operculum) areas. It is interesting to note that the orbito-frontal cortex (OFC) was not significantly activated by the samples. Examining the differences between groups, the somatosensory (SI and SII), taste (anterior-insula, mid-insula, posterior-insula, rolandic operculum) and reward areas (ACC) were activated by the samples in the pNT group. The somatosensory (SI and SII), taste (anterior-insula, mid-insula, posterior-insula, rolandic operculum, frontal operculum) and reward areas (ACC) were activated by the samples in the pMT group. The somatosensory (SI and SII) and reward areas (ACC) were significantly activated by the samples in the pST group. Only the mid-insula from the taste area was significantly activated in the pST group, showing limited activation in the taste areas as the anterior insula, posterior insula, rolandic operculum or frontal operculum were not significantly activated.

Table 6.2: Summary of brain areas significantly activated by 'all' samples. pNT = PROP non-tasters, pMTs = PROP medium-tasters, pST = PROP super-tasters.

	PROP taster groups		
Area	**pNTs**	**pMTs**	**pSTs**
Primary somatosensory (SI)	✓	✓	✓
Secondary somatosensory (SII)	✓	✓	✓
ACC	✓	✓	✓
Anterior insula	✓	✓	✗
Mid insula	✓	✓	✓
Posterior insula	✓	✓	✗
Rolandic Operculum	✓	✓	✗
Frontal Operculum	✗	✓	✗
Parietal gyrus	✓	✓	✓
Precentral gyrus	✓	✓	✓
Postcentral gyrus	✓	✓	✓
Temporal gyrus	✓	✓	✓
Frontal gyrus	✓	✓	✓
Cerebelum	✓	✓	✓
Cingulate	✓	✗	✓
Amygdala	✓	✗	✗
Occipital gyrus	✗	✗	✓

6.3.1.4 Region of Interest analysis

Specific regions of interest (ROI) were anatomically defined in the SI, SII, anterior-insula, mid-insula, posterior-insula, thalamus, ACC, OFC-lateral, OFC-medial and amygdala. Masks were created of each of these regions and the mean T-scores interrogated for each group and each stimulus using an in-house Matlab program written by Dr. Susan Francis. Results are shown in **figures 6.18 a, b and c.**

Figure 6.18 (a) shows the results for the pNT group. The high CO_2 sample increased the mean T-score in the somatosensory areas (SI, SII), mid insula, posterior insula, the thalamus and the

amygdala. The main taste area, (anterior insula) and the ACC seemed relatively unaffected by CO_2. Activation in the lateral OFC was just above 0 and activation in the medial OFC was below 0 and therefore ROI analysis shows that it was not activated by the samples in this study.

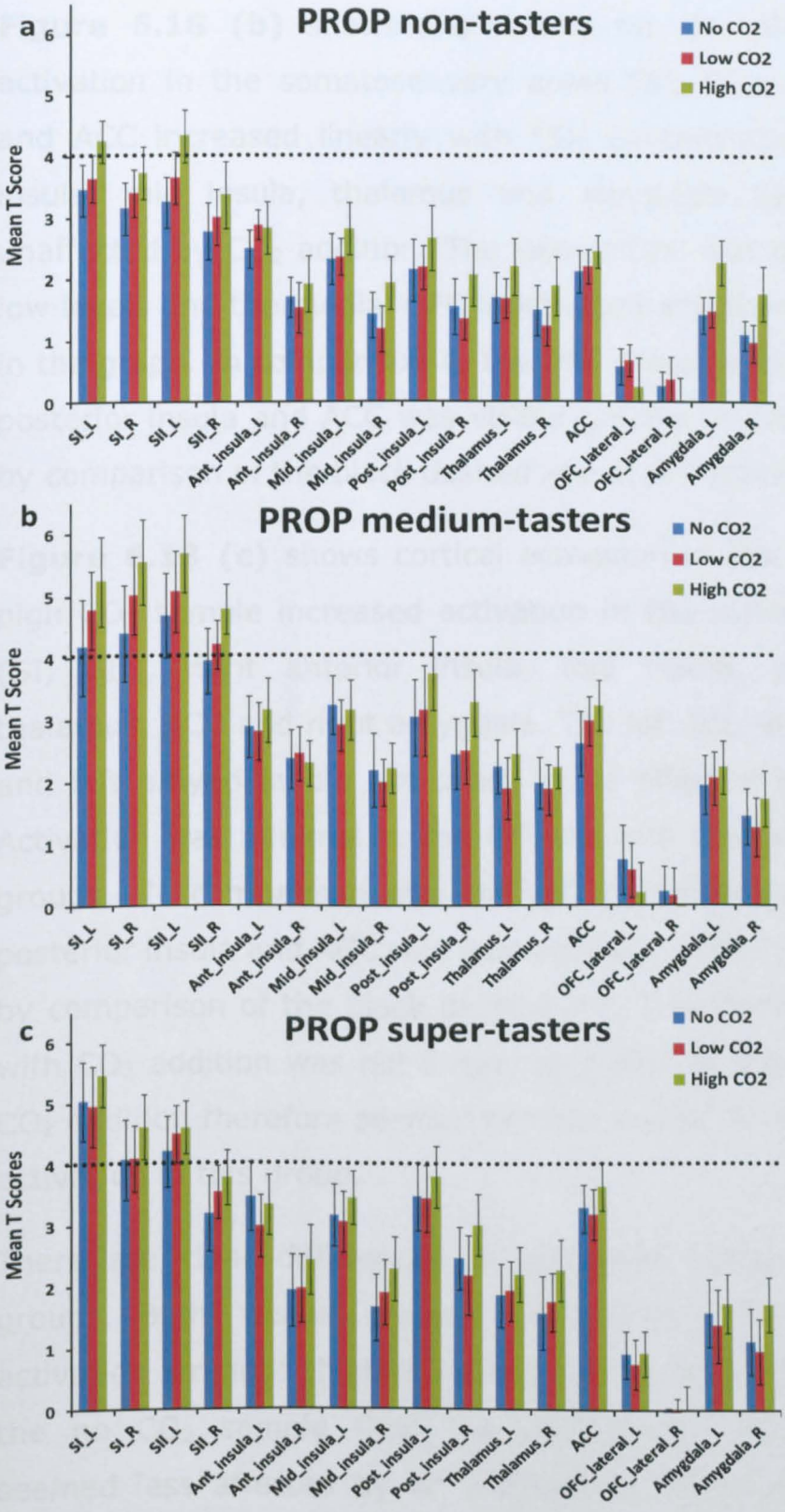

Figure 6.18 a, b and c: The mean T-scores (BOLD activation strength) from regions of interest and each sample (no CO2, low CO2 and high CO2) in (a) the PROP non-taster group, (b) the PROP medium-taster group and (c) the PROP super-taster group. Error bars show ± 1 standard error. The black dashed line indicates a T-score of 4, for ease of comparison between taster groups.

Figure 6.18 (b) shows the results for the pMT group. Mean activation in the somatosensory areas (SI, SII), posterior insula and ACC increased linearly with CO_2 concentration. The anterior insula, mid insula, thalamus and amygdala seemed relatively unaffected by CO_2 addition. The lateral OFC was activated at very low levels and the medial OFC inactivated and therefore not shown in the graph. In comparison to the pNT group, activation in SI, SII, posterior insula and ACC was visibly greater in pMTs as illustrated by comparison of the black dashed line at a T-score of 4.

Figure 6.18 (c) shows cortical activation in the pST group. The high CO_2 sample increased activation in the somatosensory areas (SI, SII), right anterior insula, mid insula, posterior insula, thalamus, ACC and right amygdala. The left SII, left anterior insula and left amygdala did not seem to be affected by CO_2 addition. Activation was minimal in the OFC as with the other PROP taster groups. In comparison with the pNT group, activation in SI, SII, posterior insula and ACC was much greater in the pST group shown by comparison of the black dashed line. The increase in activation with CO_2 addition was not linear, as shown in the pMT group, and CO_2 addition therefore seemed to have less of an impact on cortical activation in this group.

There are clear differences in activation between the different groups. Both 'taster' groups (pMTs and pSTs) had a greater activation strength (higher T-score) in the somatosensory areas to the no CO_2 sample than the pNT group. PROP super-tasters seemed less affected by an increase in CO_2 than the other two taster groups. A two sample t-test was carried out to determine if there were significant differences in the activation strength between pNT and pMT groups for 'all' samples (no CO_2, low CO_2

and high CO_2 combined) and between pNT and pST for 'all' samples. The results revealed that the pMT group had significantly higher activation in SI than pNTs as illustrated by **figure 6.19** ($p<0.01$ uncorrected) and pSTs had significantly higher activation in right SI, left SII and ACC than pNTs as illustrated by **figures 6.20 a, b and c** respectively ($p<0.01$ uncorrected).

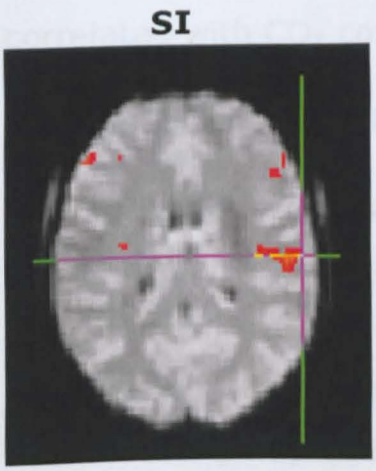

Figure 6.19: Comparison of the pMT group and the pNT group as analysed by a two sample t-test on 'all' (no CO_2, low CO_2 and high CO_2 samples combined). SI (crosshairs set at -60, -15, 20) was activated significantly greater in the pMT group.

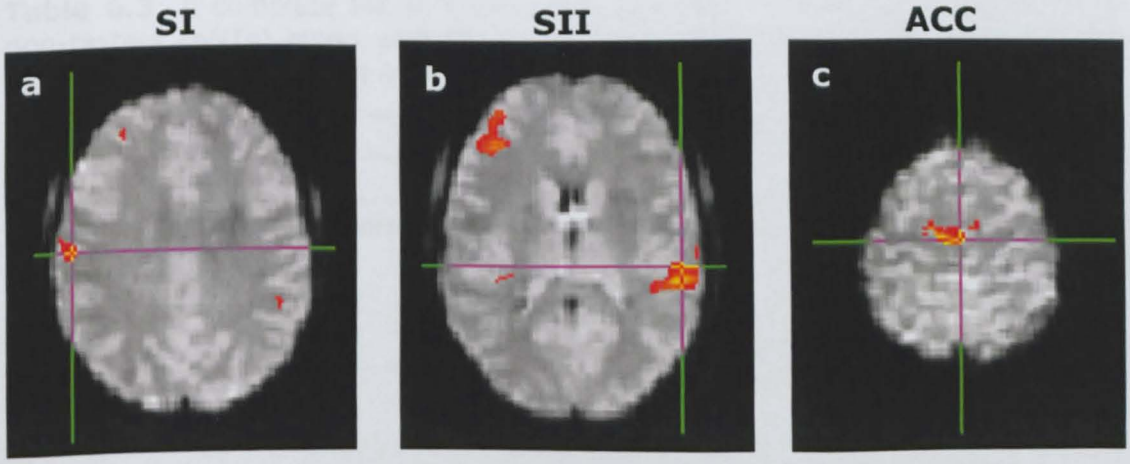

Figure 6.20: Areas activated significantly greater in the pST group compared to the pNT group analysed by a two sample t-test on 'all' (no CO_2, low CO_2 and high CO_2 samples combined). **(a)** shows significantly greater activation in SI (crosshairs at 60, -16, 28). **(b)** shows significantly greater activation in SII (crosshairs at -56, -28, 14) and **(c)** shows significantly greater activation in ACC (crosshairs at 2, -14, 62).

As shown above, there is a difference in activation strength between PROP non-tasters and PROP 'tasters' (medium and super-tasters). In addition, there also seems to be a difference between PROP 'taster' groups for their activation to increased CO_2. Activation in most ROI's in the pMT group increase linearly with CO_2 addition but this effect is not as apparent in the pST group. To investigate this relationship further, the BOLD response was correlated with CO_2 concentration and results are shown below.

6.3.1.5 Correlation of BOLD response with CO_2 concentration

The positive correlation between BOLD response and CO_2 concentration was investigated in order to determine the effect of CO_2 on brain activation. The areas of the brain showing a positive correlation of BOLD response with increasing CO_2 concentration for each PROP group are shown in **tables 6.3-6.5**, uncorrected at $p<0.001$ threshold.

Table 6.3: A complete list of brain areas positively correlated to CO_2 in PROP non-tasters (pNTs) along with their location in MNI co-ordinates, peak T-score and P-values (uncorrected <0.001)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (uncorrected)
Secondary somatosensory cortex (SII)	R	46	-14	14	4.81	0.000
		58	-18	12	4.33	0.001
Temporal gyrus (superior)	R	56	-6	4	4.57	0.000
Cerebellum	L	-16	-58	-12	4.82	0.000

Activation in the secondary somatosensory cortex (SII) was significantly positively correlated with CO_2 concentration in the pNT group revealing for the first time that CO_2 significantly increases activation in the somatosensory cortex.

Table 6.4: A complete list of brain areas positively correlated to CO_2 in PROP medium-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (uncorrected <0.001)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (uncorrected)
Primary Somatosensory Cortex (SI)	L	-56	-14	28	3.28	0.001
		-56	-12	20	3.19	0.001
Secondary Somatosensory Cortex (SII)	R	56	-6	10	5.28	0.000
	L	-54	-20	18	3.58	0.000
Posterior insula	L	-46	-28	14	3.89	0.000
Temporal gyrus (superior)	R	58	-32	16	4.19	0.001
	L	-48	-36	20	8.45	0.000
		-60	-46	20	6.75	0.000
		-64	-32	20	6.45	0.000
Temporal gyrus (mid)	R	52	-68	12	5.27	0.000
		64	-56	10	5.22	0.000
		52	-60	2	4.77	0.000
	L	-48	-66	8	3.98	0.000
		-48	-54	12	3.52	0.000
		-58	-52	12	3.33	0.000
Cerebellum	L	-34	-62	-20	4.96	0.000
SupraMarginal	R	62	-26	20	5.25	0.000
Fusiform	R	38	-54	-14	4.7	0.000

Activation in both the primary (SI) and secondary somatosensory (SII) cortices were significantly positively correlated with CO_2 concentration in the pMT group. This group also showed significant increased activation with increasing CO_2 level in additional areas compared to pNTs.

Table 6.5: A complete list of brain areas positively correlated to CO_2 in PROP super-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (uncorrected <0.001)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (uncorrected)
Thalamus	L	-16	-22	6	4.91	0.000
		-10	-28	2	4.41	0.001
Occipital gyrus (mid)	R	40	-74	12	5.15	0.000
		38	-74	4	4.87	0.000
Cerebellum	L	-18	-52	-24	6.41	0.000

Interestingly, there was no significant increase in activation in the pST group in the somatosensory or taste areas with increasing CO_2 level suggesting that this group are less affected by increasing CO_2 in those areas, which may arise due to the higher baseline activity (no CO_2) resulting in saturation of the response in these areas, as will be discussed later.

To investigate any negative correlation with CO_2 on brain activation, BOLD response and CO_2 concentration were investigated for negative correlations. Both pNT and pMT groups did not show any brain areas which were significantly negatively correlated with increasing CO_2 concentration. The pST group showed that activation in the triangular part of the inferior frontal gyrus was reduced with increasing CO_2 concentration (**table 6.6**).

Table 6.6: Brain areas negatively correlated with CO_2 in PROP super-tasters along with their location in MNI co-ordinates, peak T-score and P-values (uncorrected <0.001)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (uncorrected)
Frontal gyrus (Inferior triangular)	R	52	38	8	4.64	0.000
		44	30	16	4.55	0.000

6.3.2 Effect of thermal taster status on cortical activity

6.3.2.1 Sample preference

Figures 6.21 a, b: shows the preference of CO_2 in percentage values for thermal tasters and thermal non-tasters respectively. There is a clear difference in the pattern of response between the TT and the TnT group. The TT group most preferred the no CO_2 sample and least preferred the high CO_2 sample. In contrast, the group of TnTs analysed here did now show a preference for any

sample, with preference being spread across each of the samples. The 'no CO_2' sample was both most preferred and least preferred by the same number of subjects in this group.

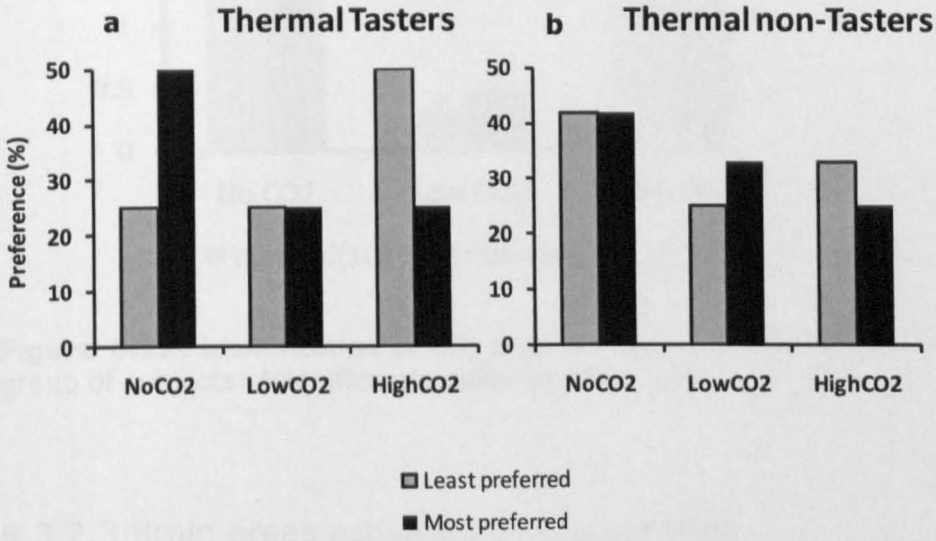

Figure 6.21: Percentage of subjects' sample preference in each group

6.3.2.2 CO_2 discrimination

Due to a malfunction, 2 of the TTs responses and 2 of the TnTs button responses were not saved resulting in 10 x full sets of TT and TnT responses for CO_2 identification. **Figure 6.22** shows that both TTs and TnTs had good levels of discrimination ability when the sample was uncarbonated. This discrimination ability was reduced for the 'low CO_2' sample and was similar between groups. However, there was a significant difference between groups for the high CO_2 sample. TTs could correctly identify the high CO_2 sample significantly more than the TnTs ($p<0.05$). It should be noted that the high CO_2 sample was least preferred by the TT group.

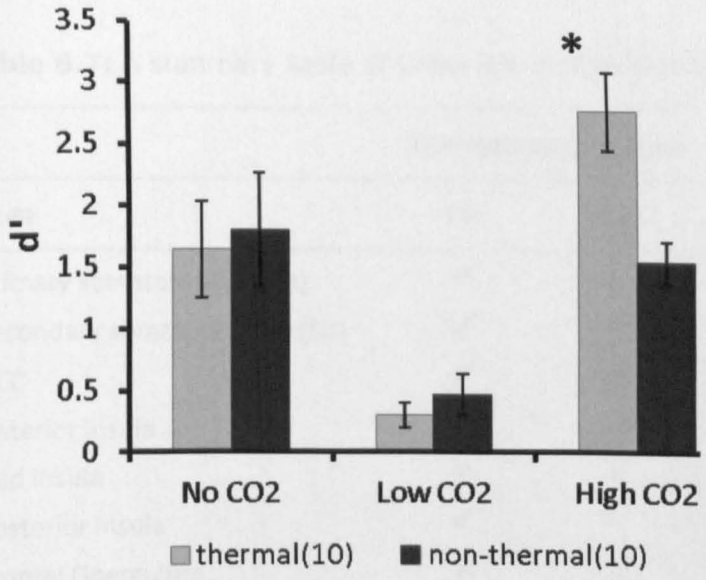

Figure 6.22: Identification of CO_2 level during scanning. Mean d' value of each group of subjects. *significantly different at $p<0.05$

6.3.2.3 Brain areas activated by the samples

The random effects group analysis (RFX) for each of the thermal taster groups revealed the areas of the brain significantly activated in response to 'all' (no CO_2 + low CO_2 + high CO_2) samples. A complete list of these brain areas, along with their location in MNI co-ordinates, T-scores and P value corrected for false discovery rate (FDR) <0.05 can be found in **appendix 3**. A summary table of the brain areas significantly activated by each group is shown in **table 6.7**. Activation was found in the somatosensory and taste areas. It is interesting to note that the orbito-frontal cortex was not activated by the samples. The TT group did not show significant activation in the anterior insula, mid insula, or frontal operculum which are considered to be taste areas in the brain. In contrast, the TnT group did show significant activation in these areas.

Table 6.7: A summary table of brain areas significantly activated by 'all' stimuli

	Thermal taster groups	
Area	**TTs**	**TnTs**
Primary somatosensory (SI)	✓	✓
Secondary somatosensory (SII)	✓	✓
ACC	✓	✓
Anterior insula	✗	✓
Mid insula	✗	✓
Posterior insula	✓	✓
Frontal Operculum	✗	✓
Parietal gyrus	✗	✓
Precentral gyrus	✓	✓
Temporal gyrus	✓	✓
Frontal gyrus	✓	✓
Cerebellum	✓	✗

6.3.2.4 Region of Interest analysis

Specific *a priori* regions of interest (ROI) were anatomically defined and their T-scores interrogated as previously described in section 6.2.8.1. Results are shown in **figures 6.22 a and b**.

Figure 6.23a shows the results for the TT group. Activation seemed relatively unaffected by increasing CO_2 concentration. In fact a trend of decreasing activation in the anterior and mid insula can be seen. Activation strength is lower in the thermal non-taster group (**figure 6.23b**) but this group appear to have more capacity for brain areas to be modulated by increasing CO_2 levels. The high CO_2 sample increased activation most notably in the somatosensory areas, the mid insula and the posterior insula.

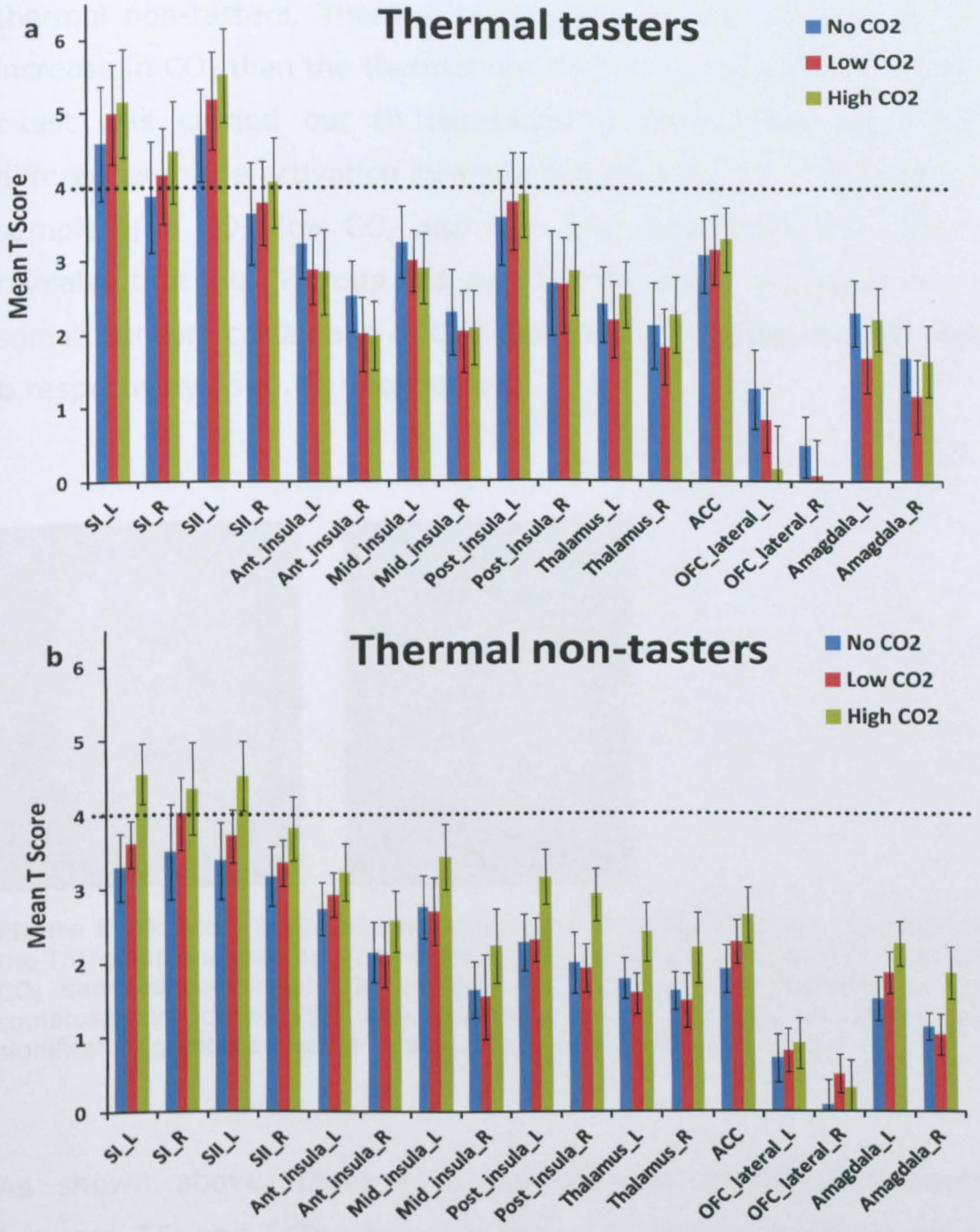

Figure 6.23 a and b: The mean T-scores (BOLD activation strength) from regions of interest and each sample (no CO2, low CO2 and high CO2) in (a) Thermal tasters, (b) thermal non-tasters. Error bars show ± 1 standard error. The black dashed line allows for easy visual comparison of activation strength between the two groups.

There are clear differences in activation between the groups. Thermal tasters have higher activation strength (T-scores) than

thermal non-tasters. Thermal tasters appear less affected by an increase in CO_2 than the thermal non-tasters groups. A two sample t-test was carried out to determine if there were significant differences in the activation strength between TTs and TnTs for 'all' samples (no CO_2, low CO_2 and high CO_2 combined). The results revealed that the TT group had significantly higher activation in the somatosensory cortex and ACC as illustrated by **figure 6.24 a and b** respectively ($p<0.01$ uncorrected).

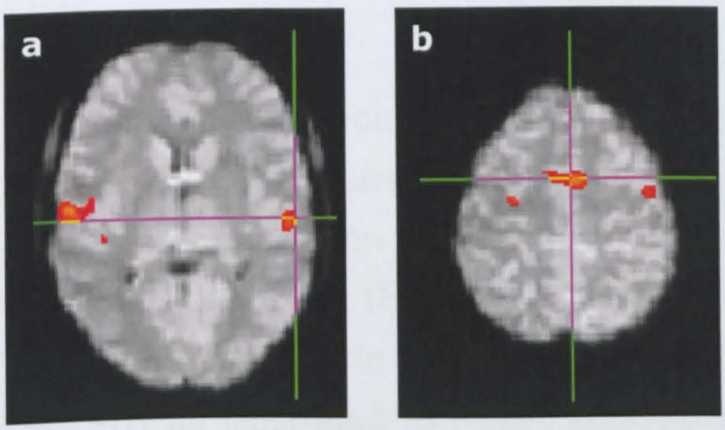

Figure 6.24: Areas activated significantly greater in the TT group compared to the TnT group analysed by a two sample t-test on 'all' (no CO_2, low CO_2 and high CO_2 samples combined). **(a)** shows significantly greater activation in the somatosensory cortex (SI) with crosshairs set at 58, -20, 10. **(b)** shows significantly greater activation in ACC with crosshairs set at 2, -2, 54.

As shown above, there is a difference in activation strength between TTs and TnTs which appears to be driven by differences in activation to the no CO_2 sample. A two sample t-test comparing TTs and TnTs in their activation to the no CO_2 sample was carried out and the results show that activation was significantly greater to the no CO_2 sample in SII and ACC in the TT group as illustrated by **figure 6.25**.

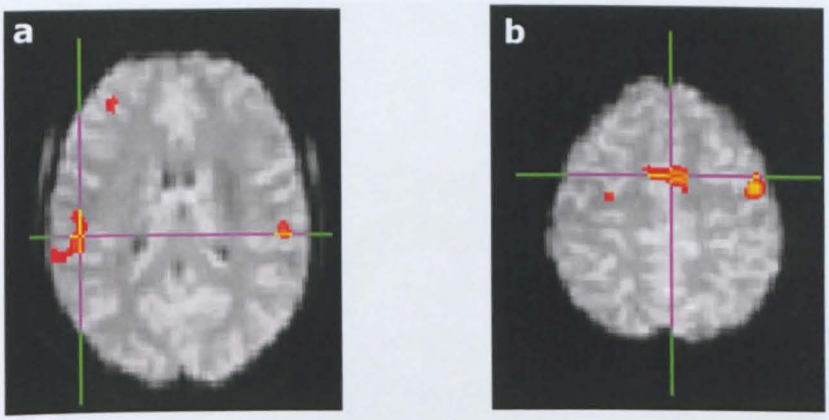

Figure 6.25: Areas activated significantly greater in the TT group compared to the TnT group analysed by a two sample t-test for the no CO_2 sample. **(a)** shows significantly greater activation in the SII with crosshairs set at 54, -26, 20. **(b)** shows significantly greater activation in ACC with crosshairs set at -2, -2, 52.

Furthermore, the groups appear to differ in their activation to increasing CO_2. A paired t-test was carried out to determine the difference in activation strength within groups to the high CO_2 and the no CO_2 samples. The thermal taster group showed significantly increased activation in small clusters in the somatosensory areas (SI, SII) as illustrated in blue in **figure 6.26**. The thermal non-taster group showed much larger clusters of activation in the somatosensory areas (SI, SII) and also in additional areas (i.e. ACC) as illustrated in red/yellow in **figure 6.26**.

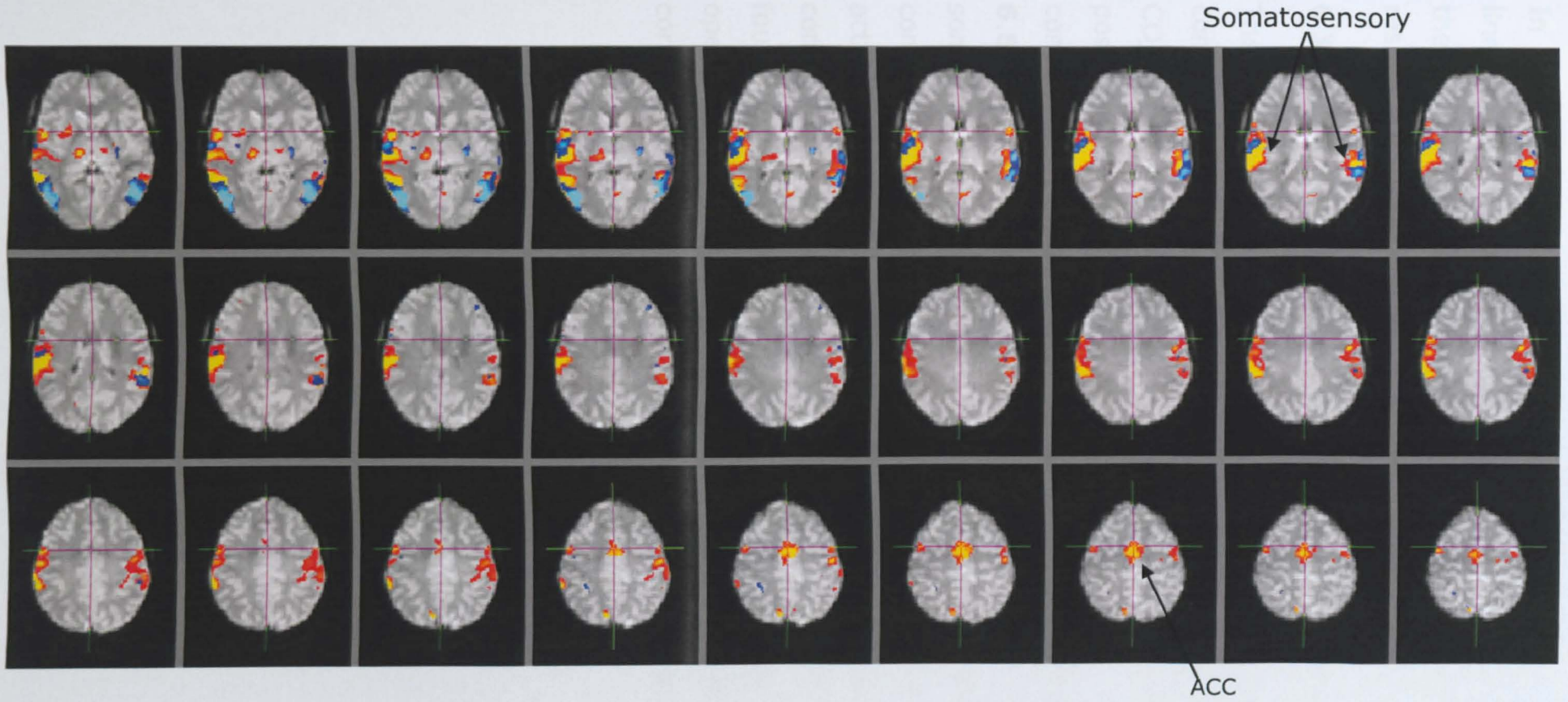

Figure 6.26: A series of axial slices with areas of increased activation to the high CO_2 sample overlaid onto an anatomical image template. Activation for the thermal non-taster (TnT) group is shown in red/yellow and activation in the thermal taster (TT) group is shown in blue. The image illustrates that TnTs had greater activation to the high CO_2 sample compared to the no CO_2 sample and this difference was much greater than that of the TT group.

In addition, activation in most ROI's in the TnT group increase linearly with CO_2 addition. To investigate this relationship further, the BOLD response was correlated with CO_2 concentration and results are shown below.

6.3.2.5 Correlation of BOLD response with CO_2 concentration

The positive correlation between BOLD response and CO_2 concentration was calculated in order to investigate the effect of CO_2 on brain activation further. The areas of the brain showing a positive correlation of BOLD response with increasing CO_2 concentration for each thermal taster group are shown in **tables 6.8-6.9**, uncorrected at $p<0.001$ threshold. The secondary somatosensory cortex (SII) was positively correlated with CO_2 concentration in thermal tasters. More areas showed increased activation with increasing CO_2 concentration in thermal non-tasters compared to thermal tasters. Significant positive correlation was found in the somatosensory areas, postcentral gyrus, rolandic operculum and the ACC. No areas were significantly negatively correlated with CO_2 level in either of the thermal taster groups.

Table 6.8: A complete list of brain areas positively correlated with CO_2 level in thermal tasters, along with their location in MNI co-ordinates, peak T-score and P-values (uncorrected <0.001)

		MNI co-ordinates				
Area	Side	x	y	z	T	P (uncorrected)
Secondary Somatosensory cortex (SII)	L	-62	-28	16	5.41	0.000
Temporal Mid	L	-46	-68	8	7.28	0.000
		-46	-60	10	5.42	0.000
	R	54	-62	0	4.39	0.001
fusiform gyrus	R	44	-54	-18	4.8	0.000
		34	-58	-10	4.41	0.001
Occipital (mid)	R	52	-78	4	5.99	0.000
		40	-72	12	5.4	0.000
		44	-78	8	5.23	0.000
Occipital (inferior)	L	-40	-64	-4	4.91	0.000
Cerebellum	L	-34	-64	-22	5.62	0.000
	L	-8	-42	-34	6.38	0.000

Table 6.9: A complete list of brain areas positively correlated with CO_2 level in thermal non-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (uncorrected <0.001)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (uncorrected)
Primary Somatosensory Cortex (SI)	R	62	-12	42	5.13	0.000
	L	-52	-14	46	5.19	0.000
		54	-16	22	3.35	0.000
		58	-20	26	3.23	0.001
Secondary Somatosensory Cortex (SII)	R	62	-26	18	8.18	0.000
		68	-28	8	3.89	0.000
postcentral gyrus	R	66	-26	38	6.27	0.000
		68	-20	32	5.06	0.000
Rolandic oppercullum	R	56	0	6	7.53	0.000
	L	-56	2	14	5.01	0.000
ACC	R	2	0	50	4.48	0.000
	L	-6	-10	48	4.55	0.000
Precentral Gyrus	R	60	-2	42	5.09	0.000
Temporal superior	L	-60	-42	20	5.75	0.000
	R	68	-38	16	3.5	0.000
Temporal Mid	L	64	-52	8	6.77	0.000
	R	50	-56	4	6.09	0.000
		58	-68	12	4.75	0.000
		-56	-52	12	4.68	0.000
Pareital lobe (superior)	L	64	-38	34	7.43	0.000
		66	-26	38	6.27	0.000
		-60	-38	38	4.61	0.000
		64	-38	34	7.43	0.000
		68	-28	26	4.57	0.000
Cerebellum	R	6	-82	-42	5.19	0.000
		14	-70	-50	4.91	0.000
	L	-20	-52	-18	4.95	0.000
		-4	-68	-14	4.74	0.000
		-6	-76	-16	4.64	0.000
		-12	-70	-48	4.64	0.000

Tables 6.8 and **6.9** show that TnTs had additional areas that were positivity correlated to CO_2 than TTs. The difference between the two groups is illustrated by **figure 6.27**. Significant positive correlation with CO_2 is shown in red/yellow for TnTs and in blue for TTs. Specific regions of interest (ROI) (inserts a-h) have been highlighted and enlarged with crosshairs set to MNI coordinates to illustrate the location of the areas positively correlated in each group. Inserts a and c show activation in SII in TnTs. Inserts d, f, g and h show activation in SI in TnTs. Insert e shows activation in the ACC in TnTs and insert b shows activation in SII in TTs.

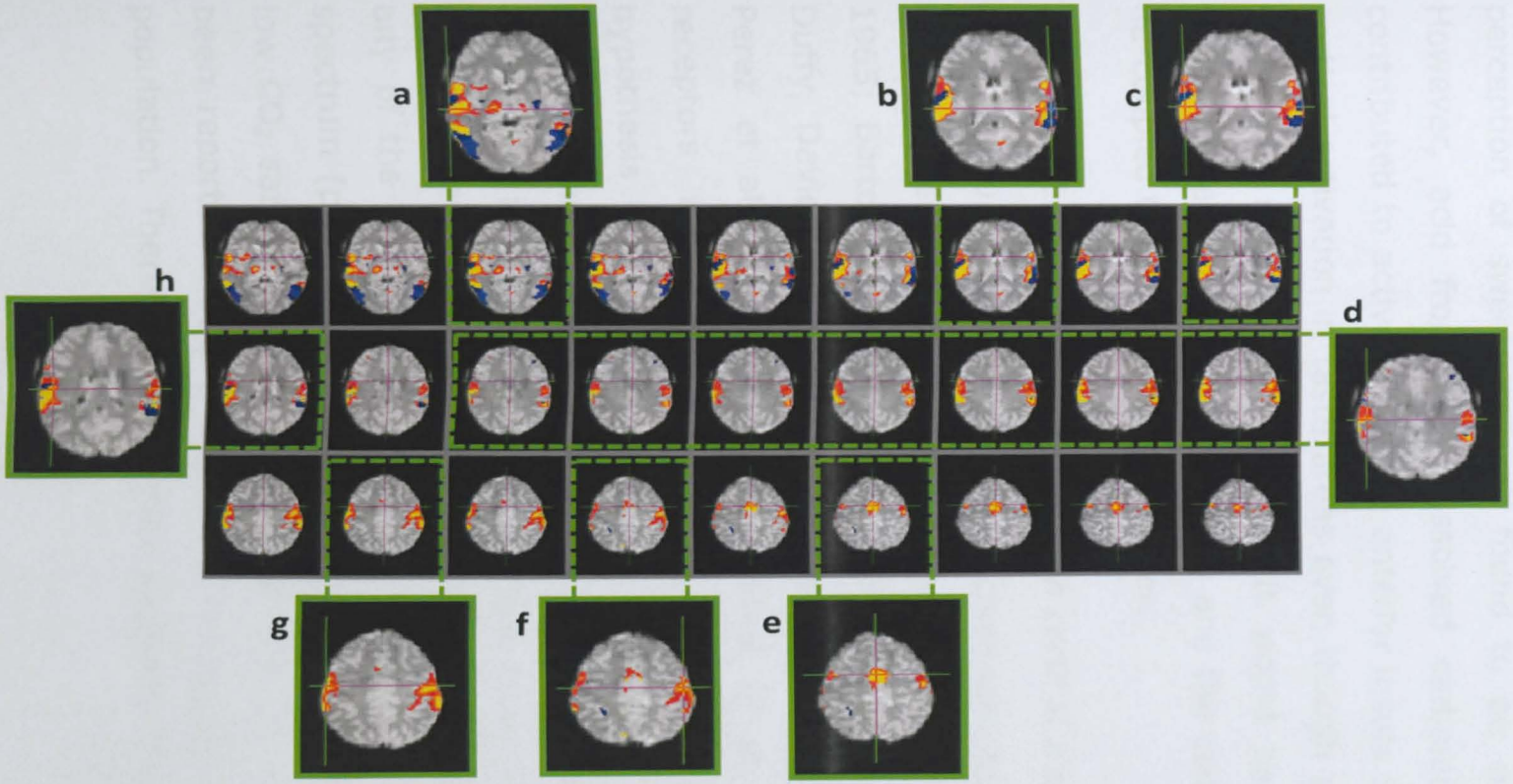

Figure 6.27: A series of axial slices with areas positively correlated to CO_2 overlaid onto an anatomical image template. Activation for the thermal non-taster (TnT) group is shown in red/yellow for the thermal taster (TT) group in blue. The image illustrates that TnTs had more areas positively correlated with CO_2 than TTs. Images a-h show larger images with crosshairs set at MNI peak coordinates from positive correlation tables 6.7 and 6.8 **(a)** crosshairs set at SII 68, -28, 8, **(b)** crosshairs set at SII -62, -28, 16, **(c)** crosshairs set at SII 62, -26, 18, **(d)** crosshairs set at SI 58, -20, 26 **(e)** crosshairs set at ACC 2, 0, 50, **(f)** crosshairs set at SI -52,-14, 46, **(g)** crosshairs set at SI 62, -12, 42, **(h)** crosshairs set at SI 54, -16, 22.

6.4 DISCUSSION

Activation in the anterior insula (part of the taste cortex) was not significantly altered by CO_2 in any of the groups; although there was a trend of negative correlation in the anterior insula in the TT group. Sensory perception results from chapter 5 would indicate that activation in taste areas may have been decreased because perception of sweetness was found to be suppressed by CO_2. However, acid from CO_2 (dissolved carbonic acid) may have contributed to activation in the anterior insula therefore increasing cortical activation in taste areas even though perception of sweet taste was significantly reduced. It would be interesting if the trigeminal effects of CO_2 (bubbles) and the carbonic acid could be de-coupled to investigate this further.

6.4.1 Effect of PROP taster status on cortical activity

Papillae count has been positively correlated to PROP taster status in a number of publications (Blakeslee 1932; Glanvill and Kaplan 1965; Bartoshuk, Duffy et al. 1994; Duffy and Bartoshuk 2000; Duffy, Davidson et al. 2004; Lanier, Hayes et al. 2005; Driscoll, Perez et al. 2006; Hayes, Bartoshuk et al. 2008). Trigeminal receptors innervate the fungiform papillae leading to the hypothesis that pSTs have more trigeminal receptors and are therefore more sensitive to trigeminal stimuli and texture, and this could lead to a preference for certain foods and beverages. This study however did not find any clear behavioural preference for any of the samples in both extremes of the PROP taster status spectrum (pNTs or pSTs). Interestingly, pMTs most preferred the low CO_2 sample and least preferred the high CO_2 sample. It has been reported that pMTs account for approximately 50% of the population. Therefore, if this data suggests that preference for CO_2

level in carbonated drinks could be within a very narrow range, then soft drink companies and breweries need to find the optimum CO_2 level for their products in order to increase preference and ultimately liking. However, preference data from much larger sample sets would be needed to confirm this.

PROP taster status did not seem to directly impact on CO_2 discrimination ability as all groups were able to adequately discriminate the high CO_2 sample, with no group showing a superior ability as may have been expected if they have more trigeminal nerves in the oral cavity as previously suggested. It is interesting to note that pMTs were the only group to show a clear preference for any of the samples and yet they seemed to have a poor discrimination ability for the sample they most preferred (low CO_2). This data would not support that PROP taster status is correlated to preference or sensitivity of trigeminal stimuli as elicited by CO_2. However, this data is unbalanced and subject numbers were low which may have influenced the outcome.

PROP 'tasters' (medium and super) tended to have overall higher trend T-scores (higher BOLD response) than pNTs in the somatosensory areas (SI, SII) anterior insula, mid insula, posterior insula and ACC, with SI, SII and ACC reaching significantly higher ($p<0.01$ uncorrected) T-scores in pSTs compared to pNTs. This increase in activation strength may be due to an increased signal from an increased number of fungiform papillae in 'tasters' compared to 'non-tasters' of PROP. Furthermore, there was a difference between groups in activation strength (BOLD response) to modulations in CO_2. PROP super-tasters did not have any regions of interest positively correlated in a linear manner with CO_2, whereas the other two groups (pNTs and pMTs) showed linear

increases in the primary somatosensory cortex (pMTs), secondary somatosensory cortex (pNTs and pMTs) and the posterior insula (pMTs). This may be because the cortical response was saturated by the unimodal taste sample (no CO_2) in pSTs due to an increased signal from an increased number of fungiform papillae. Further increases in activation in regions of interest due to CO_2 addition were therefore not possible in the pST group. This result would support an increased intensity response to taste stimuli by pSTs as previously found in this thesis (chapter 5) and in other studies (see introduction).

The addition of CO_2 did not seem to alter sample preference in the pST group with almost an equal number of subjects both 'least' and 'most' preferring each sample. When this data is considered along with the cortical activation data, it would seem possible that taste activation and preference is relatively unaffected by CO_2 addition in the pST group.

The discrimination ability of pSTs to detect the difference in CO_2 levels was not significantly different to the pNT group. Previous studies have found that pSTs have increased somatosensory acuity (Essick, Chopra et al. 2003) and that activation to oral fat in SI, SII, anterior insula, mid-insula, posterior insula was correlated with PROP taster status (Eldeghaidy, Marciani et al. 2010). These studies suggest that pSTs have an increased number of trigeminal receptors resulting in an increased sensitivity. However, this does not seem to be the case when a tastant is present as with the current study. Future research should investigate the relative contribution of taste to overall oral sensitivity in multimodal food and beverage systems.

6.4.2 Effect of thermal taster status on cortical activity

Thermal tasters seemed to have a better CO_2 discrimination ability than thermal non-tasters as they correctly identified the high CO_2 sample significantly more times than the thermal non-tasters. One hypothesis is that the innervations of trigeminal nerves in the fungiform papillae are intertwined, therefore increasing sensitivity in the oral cavity as trigeminal stimuli (temperature) are able to activate the taste nerves in this group of subjects. Therefore, taste sensations could activate the trigeminal nerves, increasing sensitivity in the oral cavity, especially when both stimuli are present. This seems plausible in light of the preference data. Thermal tasters ranked the uncarbonated (no CO_2) sample as most preferred and the high CO_2 sample as least preferred. This would suggest that the increased sensitivity of this group to trigeminal stimuli is driving preference. Thermal tasters seemed to be more sensitive to the high CO_2 sample and they least preferred this sample. In addition, the thermal taster group had higher cortical activation to the no CO_2 sample than thermal non-tasters supporting this hypothesis. Thermal tasters had overall higher T-scores than TnTs. TT also rated taste and temperature stimuli significantly higher in intensity than TnTs (chapter 5). It is therefore likely that the increase in taste and temperature intensity is a direct result of increased cortical activation in TTs.

CO_2 addition had a much greater impact on cortical activation in the TnT group than the TT group with more areas showing significant positive correlation with increasing CO_2. There was no significant negative correlation with increasing CO_2 level. However, a trend of reduced brain activation with increasing CO_2 level can be seen in the taste areas in the thermal taster group. It seems that a taste stimulus (the no CO_2 sample in this study) is activating the

brain in areas found to be correlated with taste at a very similar strength to those with an added trigeminal stimulus (the low CO_2 and high CO_2 samples). This supports the hypothesis that the taste and trigeminal nerves are intertwined at the periphery in thermal tasters. Both nerves are capable of being activated by just one chemical leading to an increased intensity response to prototypical taste stimuli and a taste response from a temperature stimulus (as shown in the previous chapter) However, the results presented here also support the central gain mechanism as proposed by Green and George (Green, Alvarez-Reeves et al. 2005). It is possible that thermal tasters have a general natural heightened ability which increases the intensity to all sensations. However, a study by Green and George (2004) found that this ability seems to be restricted to the oral cavity. Temperature intensity testing in the oral cavity was compared to the lip and hand in both TTs and TnTs. Results found that TTs rated the temperature stimuli in the oral cavity significantly more intense than TnTs but no significant differences between intensity ratings on the lip and hand were found between groups (Green and George 2004).

Investigations into thermal taster status are an exciting new area of research and the results presented here contribute to the growing body of evidence in this domain. Further research is needed to determine the mechanism for the thermal taster advantage and the hypotheses proposed here.

6.5 CONCLUSIONS AND SUMMARY

This is the first study to show the cortical response of CO_2 in the oral cavity. CO_2 activated the somatosensory areas and CO_2 concentration was found to be positivity correlated to cortical

activation in the somatosensory areas, with the strength of this activation being dependent upon the population group. Taste activation, as represented by activation in the anterior-insula, was not significantly affected by CO_2 but this could be a combinational response to both the sweet taste of the dextrose and acid from carbonic acid in the samples, thus reducing perception without significantly altering activation.

Studies investigating population differences between groups have been widely researched with focus on the difference between PROP taster status groups. Some studies have found significant differences in taste and oral somatosensation whereas others have not. This study has shown that there are differences in PROP taster groups for cortical activation of taste (sweet) and trigeminal (CO_2) stimuli. PROP non-tasters had lower overall T-scores than the pMTs and pSTs which may be explained by differences in the number and density of fungiform papillae. However, fungiform papillae counts were not investigated in this study and so this conclusion cannot be made. PROP medium-tasters had higher overall T-scores than pNTs. CO_2 addition had the greatest impact on cortical activation in pMTs as shown by positive correlation in significantly more regions of interest than pNTs. These results may be explained by an increased density in fungiform papillae and therefore trigeminal receptors in pMTs compared to pNTs. PROP super-tasters had higher overall T-scores than pNTs and less positive correlation with CO_2 than pMTs. This could be explained again by a higher number of fungiform papillae resulting in a saturation of response to the no CO_2 sample so that a further increase in activation with CO_2 is not possible in this group.

The discovery of thermal tasters is an exciting new area of research. Thermal tasters respond to taste and temperature stimuli more intensely that TnTs (chapter 5). The mechanism for this increase in intensity has been proposed to be due to both peripheral and central mechanisms. This is the first study to investigate the difference in cortical activation to gustatory (sweet taste) and trigeminal stimuli (CO_2) between thermal and non-thermal taster groups. Thermal tasters seem to be more sensitive to trigeminal stimuli than thermal non-tasters as they were able to discriminate the high CO_2 sample significantly more than TnTs. In addition, this increased sensitivity in the TT group seemed to shape preference, as the high CO_2 sample was ranked as the least preferred sample and the no CO_2 sample was the most preferred. These findings were represented cortically with thermal tasters showing a higher cortical response to the no CO_2 (taste) sample than TnTs which may be driving preference in this group. Thermal tasters had higher overall T-scores than TnTs possibly resulting from increased activation due to interaction of taste and trigeminal nerves or from a central gain mechanism as proposed by Green and George (2005). This could also be the reason for less positive correlation of BOLD response with CO_2 addition in this group compared to the TnT group.

PROP taster status and possible correlations to overall oral sensitivity, food preference, obesity and alcoholism have been studied for over 80 years and so far evidence is inconclusive. In contrast, thermal stimulation of taste (thermal taster status) was discovered a little over 10 years ago. To date, only seven studies have been published investigating thermal taster status and evidence seems much less variable compared to research investigating PROP taster status. Dedicated research is required to

further understanding but it seems increasingly likely that thermal tasters could be the new PROP super-tasters.

Chapter 7

7. CONCLUSIONS

The primary aim of this research was to investigate the interactions between sweetness, bitterness, alcohol content and carbonation level on flavour perception. A model beer was created using sweetener (dextrose), hop acids, ethanol, CO2, aroma volatiles (simple blend of five compounds commonly found in beer), colouring and polydextrose (to give a base level of viscosity). This fundamental investigation into multimodal interactions on flavour perception will not only be of interest to researchers but also the food and beverage industry such as the alcoholic and soft drinks sectors. In particular, there has been recent interest in the development of reduced ethanol and CO_2 beers to increase market share by targeting different consumers and to reduce costs to the consumer due to increased taxation of alcohol.

A large body of research continues to focus on population differences in flavour perception and consumer attitudes towards food in terms of preference, liking, purchase intent and buying behaviour. Therefore, a secondary aim of this research was to investigate differences in population groups for their genetic sensitivity to oral stimuli.

This research has taken a multidisciplinary and systematically controlled approach to investigate multimodal flavour perception using a design of experiments comprising of instrumental flavour chemistry, descriptive sensory evaluation and functional magnetic resonance imaging.

Results found that CO_2 and ethanol both increased volatile partitioning from the breath during consumption which, when added together could contribute an 86% increase in volatile delivery to the olfactory cavity. Sensory profiling techniques

however, did not find that this increase significantly altered aroma perception sensorially. However, the mixture of volatiles in the model system was basic, comprising of just five compounds. It would be pertinent to investigate more volatiles, given the complexity and number of volatiles present in beer, to understand the true impact a reduction in ethanol and CO_2 could have on flavour perception.

Sensory analysis found ethanol to be the main driver of warming perception, complexity of flavour and also it also contributed to sweetness perception in the model beer. It would be hypothesised therefore, that if ethanol was reduced to <2.8% (the levels required to reduce taxation), then this would bring about a reduction in complexity of flavour, warming perception and possibly sweetness perception. The impact that this could have on consumer liking should be investigated by means of a consumer study. Results would give insight into which attributes are significantly affected amongst consumers and this could be used alongside the trained panel data to ensure successful product development.

CO_2 significantly affected all discriminating attributes showing an ability to interact and modify flavour perception in a complex manner. Efforts at reducing CO_2 levels in beer would benefit from this research to know what attributes are being affected, how this combines to alter flavour perception and then start to investigate ways of boosting or balancing flavour perception to match that of the standard CO_2 versions.

Successful product development is made increasingly difficult due to population differences in oral sensitivity. A reduced ethanol and CO_2 product may not appeal to someone with low oral sensitivity

but may appeal to someone with high oral sensitivity. Two markers of genetic sensitivity were investigated, PROP taster status and thermal taster status. PROP taster status is the ability to taste the compound, 6-n-propylthiouracil (PROP). Those who cannot taste the compound are classified as PROP non-tasters and those who can taste the compound are classified as PROP tasters. PROP tasters can be further split into two groups, those who taste the compound with 'medium' intensity are called PROP medium-tasters and those who taste the compound at a high intensity are called PROP super-tasters. Thermal tasting on the other hand describes an ability of thermal stimulation (to the tongue) to evoke a taste response. In contrast, thermal non-tasters do not taste anything during thermal stimulation. Volunteers were screened for their PROP and thermal tasters status. This study used a simplified method with more relaxed criteria for screening thermal tasters to those previously published and found that the simplified method was valid.

Taste and temperature intensity data were collected on each subject and found that differences in the population's oral sensitivity could be explained by PROP taster status and thermal taster status. PROP tasters gave significantly ($p<0.05$) higher intensity ratings for each stimuli than PROP non-tasters. Thermal tasters rated the intensity of the stimuli significantly ($p<0.05$) higher than thermal non-tasters. However, both PROP and thermal markers of oral sensitivity cannot be explained by the same mechanism as thermal tasters were relatively evenly distributed across PROP taster groups and vice-versa. Future research should attempt to uncover the mechanisms behind thermal taster status by first understanding if increased intensity perception is limited to oral stimuli. Studies investigating the response to aroma and other

sensory stimuli between thermal taster groups would facilitate understanding of the mechanisms responsible. Furthermore, it is currently unknown if thermal taster status has a genetic basis and so family studies should be employed to determine this.

Sensory response to CO_2 is via a dual mechanism of action; the nociceptive response (tingle) and inevitable taste from carbonic acid and the mechanical response from the presence of bubbles. It would be interesting if the two aspects could be de-coupled and investigated to further understand the contribution from each on flavour perception and cortical activation. This would help to determine the contribution of CO_2 on taste perception due to the acidic and mechanical components. CO_2 did not significantly impact activation in the primary taste areas of the brain (anterior insula) in this study. However, interesting trends in the data were found between thermal taster groups. Investigations into how CO_2 impacts on cortical activation using fMRI have for the first time shown that CO_2 significantly increases activation in the somatosensory cortex. Furthermore, there were differences in cortical activation strength in certain regions of interest between PROP taster groups and thermal taster groups which reflected the differences seen in intensity ratings of oral stimuli. The increased cortical activation in thermal tasters also seemed to explain behavioural data. Thermal tasters least preferred the high CO_2 sample and most preferred the no CO_2 (uncarbonated) sample and were significantly better discriminators of high CO_2 than the thermal non-tasters. These findings may be due to an increased sensitivity to CO_2 in thermal tasters. It is currently unknown why, and how this is happening but the hypothesis presented here is that it could be caused by a connection between the taste and trigeminal nerves in thermal tasters. This would allow a taste

response to come from a trigeminal stimulus (temperature) and similarly allowing both nerves to be activated to a taste stimulus, increasing intensity response and cortical activation and influencing preference. Future work should investigate this proposed mechanism. Animal studies have given insight into the activation of groups of neurons to certain oral stimuli. Further research investigating the peripheral biology of the taste and temperature nerves in the fungiform papillae and how this may be linked to possible differences in strength of activation from taste and temperature stimuli on the relevant neurons may begin to uncover the mechanism of thermal taster status.

There are currently only seven papers published on thermal taster status and so research in this area is very limited. Considering the implications of the results found here, future work should concentrate on the link between the thermal taster advantage and hedonic liking as is seems increasingly likely that a proportion of the population (thermal tasters) sense oral stimuli differently which could shape their liking, consumer behaviour and ultimately their health. From a sensory scientist's point of view, the possibility of screening for thermal taster status to create a trained panel of thermal tasters could increase sensitivity and reduce variability in sensory data.

7.1 FURTHER WORK

Throughout this thesis, suggestions have been made for areas of further work which are summarised below.

- Further investigations into the physico-chemical effects of ethanol and carbonation with more compounds than used in this study (chapter 3) should be carried out following a similar method. This will provide additional data to test the

hypothesis of the relationship between a compounds's K_{aw} and the effects of ethanol and carbonation on partitioning into the breath. It will also give more insight into the cumulative effects of ethanol and carbonation on aroma delivery and therefore perception.

- A study should be carried out to determine if the effects of CO_2 addition on flavour perception found in the model beer system used in this study can be applied to real beer. Standard lager beer could be de-gassed using sonication and then re-carbonated to different levels and compared for their sensory attributes.

- An investigation to determine if hop acid addition alters the bubble size of carbonated samples would establish if the interaction between hop acids and CO_2 on tingly and carbonation perception at low CO_2 levels as found in chapter 4 is due to differences in surface tension and bubble formation. Bubble size could be measured by photographing samples and measuring the bubble diameter.

- Further investigate the mechanism of the ethanol-CO_2 interactions found in this study (chapter 4) by studying if ethanol and CO_2 leads to cross-desensitisation when presented simultaneously using a similar method to Green (1991).

- The contribution of sweetness, bitterness, alcohol content and carbonation to consumer liking could be measured in a consumer study and used alongside the sensory data collected in chapter 4 to provide more information to new product developers and marketing teams.

- Investigations into the effect of hop acids on the sweet transduction cation channel, TRPM5, to determine the source of the sweetener-hop acid interaction found in chapter 4.

- Further investigations into the possible differences between PROP taster groups and thermal taster groups for sensory interactions between modalities should be explored to understand the effect of taste phenotype on more complex food and beverage systems. Specifically the relative contribution of taste on overall oral sensitivity should be explored in multimodal food and beverage systems.

- Studies investigating the intensity of olfactory perception and cutaneous pain would further understanding into the origins of the thermal taster mechanism. In addition, family studies would give indication if thermal taster status is genetic in origin.

- De-couple the mechanical aspect of CO_2 and the nociceptive carbonic acid to determine the contribution of each on flavour perception.

- CO_2 preference data from larger sample sets of each PROP taster group and both thermal taster groups would confirm if the trends found in this thesis (chapter 6) between each marker of taste sensitivity and liking of CO_2 are valid.

Appendix 1: University of Nottingham Magnetic Resonance Centre safety screening questionnaire

[SAFETY SCREENING QUESTIONNAIRE]

The University of Nottingham

Sir Peter Mansfield Magnetic Resonance Centre

Safety Screening Questionnaire

For ANYONE entering the INNER CONTROLLED AREA marked by red and white tape on the doors (Magnetic field safety information is available in the SPMMRC website)

>> Shaded boxes to be filled in by scan volunteers and patients only <<

NAME	Date of Visit	Phone Number
ADDRESS	Volunteer Number	
	Date of Birth	
Study Ethics Code (or reason for visit)	Hospital No (if applicable)	
	Weight (Philips scanners only)	

MR scanning uses strong magnetic fields, for your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear.

All information is held in the strictest confidence.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)? Y/N
3. Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)* Y/N
4. Have you ever had any surgery? Please give brief details* Y/N

*(*We do not need to know about uncomplicated caesarian delivery, vasectomy or termination of pregnancy)*

5. Do you have any foreign bodies in your body (e.g. shrapnel)? Y/N
6. Have you ever worked in a machine tool shop without eye protection? Y/N
7. Do you wear a hearing aid or cochlear implant? Y/N
8. Could you be pregnant? You must use the pregnancy tests available in the female toilets if you are unsure. Y/N
9. Have you ever suffered from tinnitus? Y/N
10. Do you wear dentures, a dental plate or a brace? Y/N
11. Are you susceptible to claustrophobia? Y/N
12. Do you suffer from blackouts, epilepsy or fits? Y/N
13. Do you have any trans-dermal patches (skin patches)? Y/N

6. Do you have any tattoos? Y/N

7. Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing aids etc before entering the magnet hall.? *(lockers available by the changing rooms)* Y/N

16. Is there anything else you think we should know? Y/N

I have read, understood, and answered all questions **Signature:**	**Date:**
Verified by: **SPMMRC Staff Signature:**	**Date:**

Appendix 2: Brain areas activated by stimuli in PROP taster groups

Table 2.1: A complete list of brain areas (above 10 in cluster size) activated by all stimuli in PROP non-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (FDR corrected <0.05).

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (FDR)
Primary Somatosensory Cortex (SI)	R	60	-16	28	13.15	0.000
		60	-6	26	11.27	0.000
		50	-8	38	9.69	0.000
	L	-52	-14	24	13.36	0.000
		-58	-10	30	12.93	0.000
Secondary Somatosensory Cortex (SII)	R	60	-16	12	17.17	0.000
		64	-10	22	11.58	0.000
		52	-22	14	11.41	0.000
	L	-66	-20	16	9.4	0.000
ACC	R	6	-4	52	13.41	0.000
		4	-10	60	12.42	0.000
		0	6	52	12.04	0.000
	L	-6	-6	64	10.01	0.000
Anterior_insula	R	36	12	0	12.51	0.000
		40	10	-4	11.87	0.000
	L	-38	18	-14	5.9	0.000
Mid_insula	R	46	0	-4	9.94	0.000
posterior insula	R	38	-10	8	10.17	0.000
Rolandic operculum	R	62	6	10	10.77	0.000
		52	2	8	9.27	0.000
Parietal_sup	L	-24	-58	68	4.73	0.001
Precentral Gyrus	R	36	-14	44	12.02	0.000
Postcentral	R	42	-24	42	9.65	0.000
	L	-52	-20	46	10.43	0.000
Temporal_sup	R	64	-38	16	10.89	0.000
		62	-10	6	10.39	0.000
		52	4	-12	10.01	0.000
		56	6	-6	9.62	0.000
	L	-52	12	-14	6.5	0.000
		-44	12	-18	6.13	0.000
Frontal gyrus (mid)	L	-44	44	20	8.05	0.000
		-36	48	24	7.34	0.000
		-40	38	34	5.63	0.000
Cerebelum	R	20	-52	-18	14.02	0.000
	L	-16	-68	-18	12.95	0.000
Cingulate/calcarine	R	10	-58	-14	15.72	0.000
Cingulate/cingulum	R	6	12	42	9.9	0.000
amygdala/paraHippocampus	L	-20	6	-24	6.43	0.000
		-18	-2	-24	5.7	0.000

Table 2.2: A complete list of brain areas (above 10 in cluster size) activated by all stimuli in PROP medium-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (FDR corrected <0.05).

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (FDR)
Primary Somatosensory Cortex (SI)	R	60	-8	26	8.27	0.000
	L	-50	-12	32	7.95	0.000
Secondary Somatosensory Cortex (SII)	R	66	-12	22	8.84	0.000
		64	-12	10	7.63	0.000
	L	-58	-6	22	9.43	0.000
		-54	-22	16	9.07	0.000
ACC	R	10	-8	58	8.93	0.000
		-4	-8	56	11	0.000
		-38	12	-2	8.67	0.000
Anterior_insula	L	-38	12	-2	8.67	0.000
Mid_insula	R	50	0	-4	13.18	0.000
posterior insula	R	32	-28	10	8.91	0.000
		36	-8	14	8.03	0.000
	L	-34	-14	16	7.76	0.000
Rolandic operculum	L	-52	-6	8	8.93	0.000
Frontal_operculum	R	58	10	22	11.16	0.000
		56	12	12	9.01	0.000
Frontal (inferior)	R	44	36	6	7.5	0.000
	L	-44	40	10	5.09	0.000
Frontal gyrus (mid)	L	-40	30	30	7.29	0.000
Parietal_inf	R	54	-38	56	8.07	0.000
Precentral Gyrus	R	44	-16	40	8.95	0.000
		48	-14	40	8.87	0.000
		50	0	42	8.5	0.000
	L	-58	0	26	9.1	0.000
		-38	-22	66	7.95	0.000
Postcentral	R	50	-10	22	9	0.000
		52	-18	58	8.97	0.000
	L	-42	-16	44	9.22	0.000
		-46	-14	40	8.88	0.000
Temporal_sup	L	-44	10	-18	5.82	0.000
		-54	6	-16	5.82	0.000
		-54	-4	4	8.86	0.000
Temporal_mid	R	46	-20	-10	7.61	0.000
Temporal_inf	L	-52	-38	-16	7.65	0.000
Cerebelum	R	18	-58	-26	7.88	0.000

Table 2.3: A complete list of brain areas (above 10 in cluster size) activated by all stimuli in PROP super-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (FDR corrected <0.05).

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (FDR)
Primary Somatosensory Cortex (SI)	R	62	-12	18	10.9	0.000
		58	-14	46	10.69	0.000
Secondary Somatosensory Cortex (SII)	R	56	-6	22	8.71	0.000
	L	-64	-22	20	9.26	0.000
ACC	R	10	-4	50	11.12	0.000
		10	4	46	9.14	0.000
	L	-4	8	50	9.56	0.000
		-6	6	54	9.52	0.000
Cingulate	R	10	14	42	9.8	0.000
		8	16	38	9.78	0.000
Mid_insula	R	50	-2	0	9.81	0.000
Fontal_sup	R	28	-10	60	8.82	0.000
		20	-12	66	8.59	0.000
Frontal (inferior)	L	-42	44	8	4.86	0.000
Frontal gyrus (mid)	L	-40	40	22	5.52	0.000
		-30	50	18	4.99	0.000
Parietal_inf	L	-54	-36	44	9.78	0.000
		-40	-48	42	8.88	0.000
		-42	-50	46	8.78	0.000
Precentral Gyrus	R	42	-8	60	10.03	0.000
		30	-16	54	9.54	0.000
Postcentral	R	68	-14	24	11.17	0.000
		50	-24	38	8.83	0.000
	L	-52	-28	52	10.75	0.000
		-50	-22	42	9.71	0.000
Temporal_sup	R	62	-24	8	8.95	0.000
Lingual (Occipital lobe)	R	20	-72	-10	9.24	0.000
Occipital_mid (cuneus)	L	-14	-102	8	14.33	0.000
		-18	-94	6	11.6	0.000
		-16	-98	-8	9.03	0.000
		-16	-100	-2	9.31	0.000
Calcarine	R	22	-94	0	10.73	0.000
Cerebelum	R	12	-62	-16	11.27	0.000
	L	-42	-62	-28	8.91	0.000
SupraMarginal	R	56	-28	24	10.11	0.000

Appendix 3: Brain areas activated by stimuli in thermal taster groups

Table 3.1: A complete list of brain areas (above 10 in cluster size) activated by all stimuli in thermal tasters, along with their location in MNI co-ordinates, peak T-score and P-values (FDR corrected <0.05)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (FDR)
Primary Somatosensory Cortex (SI)	R	50	-14	44	14.16	0.000
		58	-6	32	10.7	0.000
		48	-12	32	9.82	0.000
	L	-48	-12	44	11.94	0.000
Secondary Somatosensory Cortex (SII)	R	62	-14	12	11.5	0.000
		58	-10	4	8.93	0.000
		54	-30	18	9.12	0.000
	L	-62	-22	18	7.93	0.000
		-56	-8	24	7.87	0.000
ACC	R	10	-8	56	10.89	0.000
		8	6	42	8.82	0.000
	L	-2	-6	54	16.16	0.000
		-4	-10	64	10.86	0.000
		-4	2	48	10.05	0.000
posterior insula	L	-44	-12	8	8.81	0.000
Frontal opercullum	R	58	8	24	10.51	0.000
Precentral Gyrus	R	42	-18	40	11.62	0.000
		56	2	44	8.68	0.000
		52	2	46	8.68	0.000
	L	-28	-18	64	9.37	0.000
		-44	-2	54	8.26	0.000
		-40	-4	58	8.19	0.000
Frontal gyrus (inferior)	L	-42	44	14	4.2	0.000
Frontal gyrus (mid)	R	38	48	22	8.39	0.000
		34	40	30	8	0.000
	L	-38	32	28	5.04	0.000
		-40	48	22	4.75	0.000
Temporal Superior	R	54	2	-4	11.54	0.000
	L	-44	10	-18	6.86	0.000
ParaHippocampal	L	-8	-28	-12	8.73	0.000
		-18	4	-26	3.04	0.000
Precuneus	R	10	-64	-22	8.32	0.000
Cerebelum	R	22	-54	-26	9.96	0.000
		36	-54	-38	9.5	0.000
vermis_6	L	-2	-70	-14	7.92	0.000
red nucleus/parahippocampus	R	4	-26	-8	9.2	0.000
red nucleus	L	-2	-26	-6	9.08	0.000

Table 3.2: A complete list of brain areas (above 10 in cluster size) activated by all stimuli in thermal non-tasters, along with their location in MNI co-ordinates, peak T-score and P-values (FDR corrected <0.05)

		MNI peak co-ordinate				
Area	Side	x	y	z	T	P (FDR)
Primary Somatosensory Cortex (SI)	R	56	-14	20	13.97	0.000
		62	-14	22	13.76	0.000
Secondary Somatosensory Cortex (SII)	R	64	-22	10	15.28	0.000
		50	-10	10	13.9	0.000
		60	-14	10	11.71	0.000
		56	-32	18	10.44	0.000
	L	-62	-12	10	13.39	0.000
		-60	-6	18	11.28	0.000
		-52	-10	12	11.04	0.000
		-58	-20	14	11.98	0.000
		-58	-28	6	10.48	0.000
ACC		0	-8	58	14.27	0.000
Anterior Insula	R	32	16	-4	12.95	0.000
	L	-38	8	0	11.71	0.000
		-44	14	-2	10.81	0.000
Mid Insula	R	46	2	-4	23.55	0.000
Posterior Insula	L	-42	-24	-4	10.71	0.000
		-36	-12	4	10.99	0.000
		-36	-16	8	10.45	0.000
		-32	-22	6	10.41	0.000
		-46	-12	16	10.3	0.000
Precentral Gyrus	R	52	0	20	10.97	0.000
		44	-2	40	10.58	0.000
Frontal operculum (inferior)	R	56	10	20	15.68	0.000
Frontal gyrus (inferior)	L	-46	36	22	15.65	0.000
		-42	40	12	8.3	0.000
Frontal gyrus (mid)	L	-34	48	18	6.89	0.000
Temporal superior	R	54	8	-8	11.18	0.000
	L	-50	4	-4	16.02	0.000
		-40	16	-16	7.37	0.000
		-54	12	-14	6.35	0.000
Temporal_mid	R	58	-58	6	10.66	0.000
	L	-64	-48	-10	11.13	0.000
Temporal Inferior	L	-62	-54	-12	10.79	0.000
Pareital lobe (superior)	L	-26	-54	70	4.81	0.000
ParaHippocampal	L	-16	6	-24	4.91	0.000
Putamen	R	24	-12	8	11.52	0.000
	L	-14	8	4	10.35	0.000

Appendix 4: Achievements

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Clark, R., Linforth, R., Bealin-Kelly, F., Hort, J. (2011) Effects of ethanol, carbonation and hop acids on volatile delivery in a model beer system. Journal of the Institute of Brewing. 117(1):74-81

Clark, R.A., Hewson, L., Bealin-Kelly, F., Hort, J. (2011) The interactions of CO_2, ethanol, hop acids and sweetener on flavour perception in a model beer. Chemosensory Perception. 4:42-54

SYMPOSIA PRESENTATIONS

Oral: Royal Chemistry Conference, Queens College, Belfast (2008)

Poster: European Brewing Congress, Hamburg (2009)

Poster: Pangborn Sensory Science Symposium, Florence (2009)

Oral: 2nd International Symposium for Young Scientists and Technologists in Malting, Brewing and Distilling, Weihenstephan, Germany (2010)

Oral: Eurosense symposium, Spain, (2010). Successful applicant of PFSG's travel award (2010)

Oral: Pangborn Sensory Science Symposium, Toronto (2011)

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